

	Q1*	Q3*	4*	5*	550	EDTA	2*	2*	410	550	Activity
	µM	µM	µM	µM	µM	µM	µM	µM	µM	µM	µg/µB
	GSH	GSSG	NaOH	KCl	PEG 3550	GdnHCl			Sucrose	Agg.	
2 (55 mM MES pH 6.5)	+	+	+	10.56	0.44	0	+	+	0	0	0
3 (55 mM MES pH 6.5)	+	+	0	10.56	0.44	+		0	+	+	0
#5 (55 mM MES pH 6.5)	+	+	0	264	11	0	+	+	+	0	0
#8 (55 mM MES pH 6.5)	+	+	+	264	11	+	0	0	0	+	40.00
#10 (55 mM Tris pH 8.2)	+	+	+	10.56	0.44	0	+	0	+	0	0
#11 (55 mM Tris pH 8.2)	+	+	0	10.56	0.44	+		+	0	+	105.26
#13 (55 mM Tris pH 8.2)	+	+	0	264	11	0	+	0	0	0	15.65
#16 (55 mM Tris pH 8.2)	+	+	+	264	11	+	+	+	+	+	48.70

Figure 1

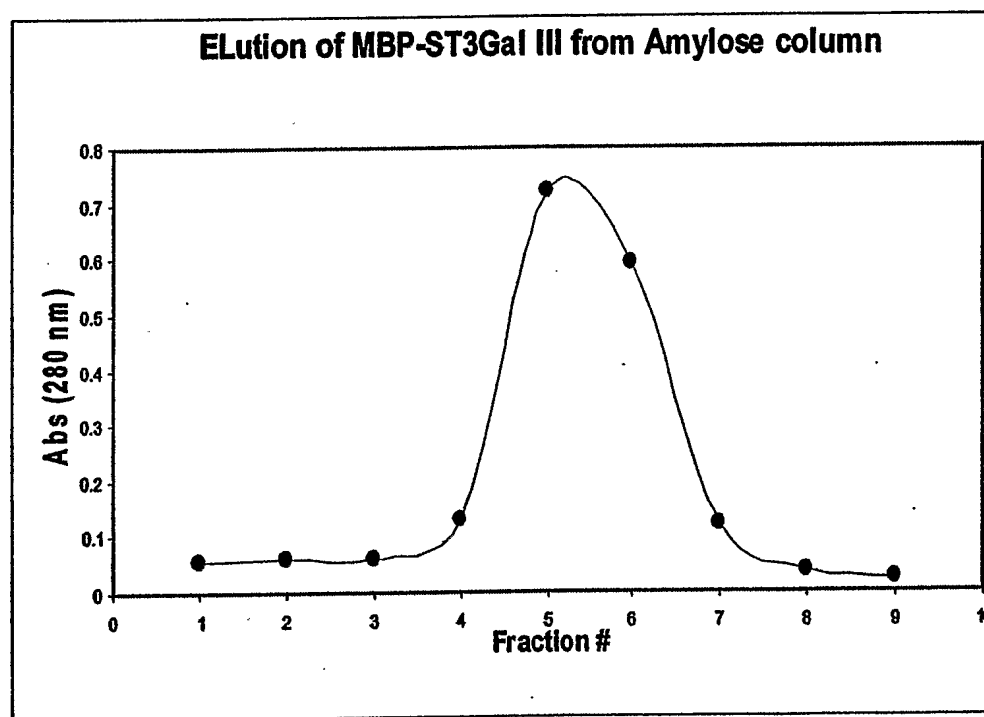


Figure 2

**ST3 Gal III activities of the Amylose purified  
refolded MBP-ST3Gal III fractions**



Figure 3

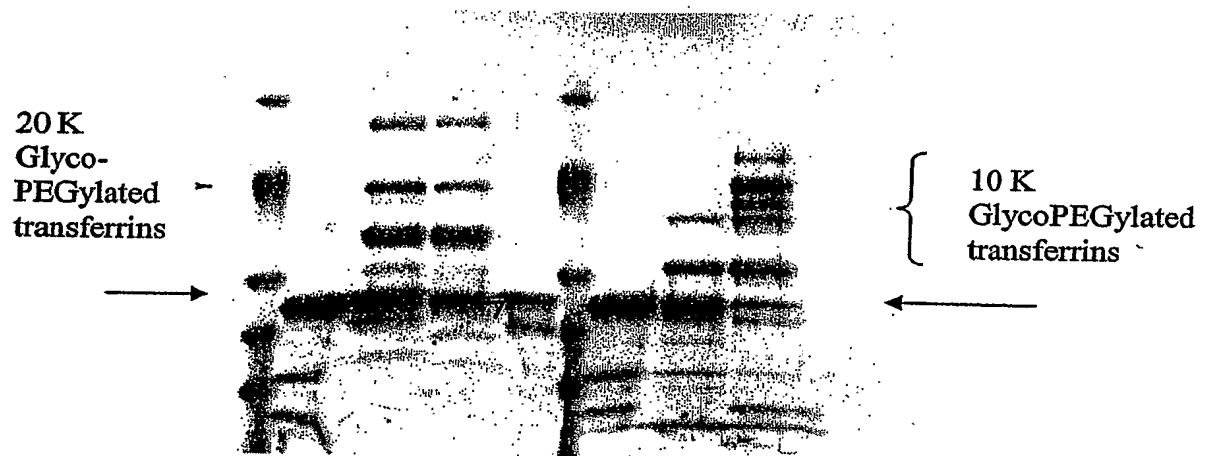
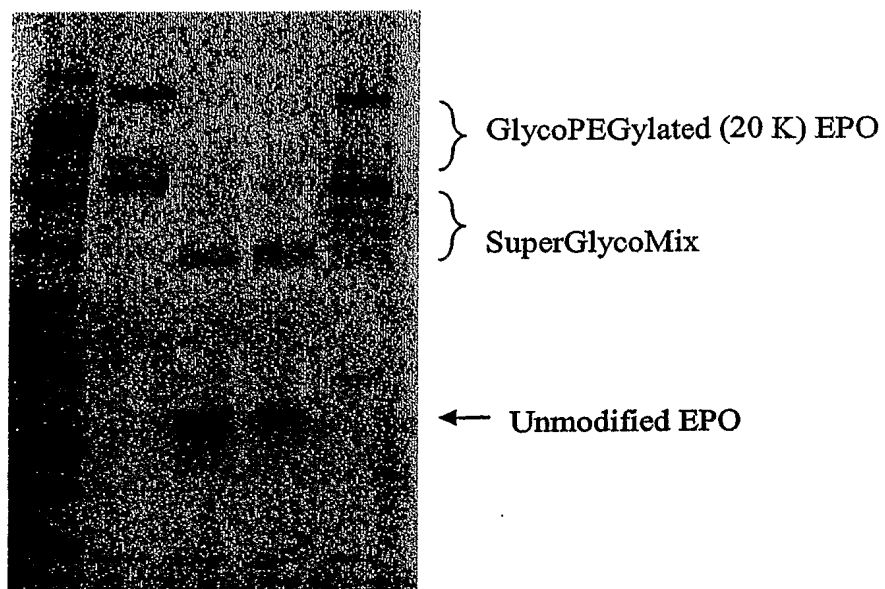


Figure 4



**Figure 5. GlycoPEGylation (20 K) of EPO**

**Figure 5**

```

      0   10   20   30   40   50   60
/usr/t MLKKQSAGLVLWGAILFVAWNALLLLFFWTRPAPGRPPSVSALDGDGPASLTREVIRLAQD
       :::::::::::::::::::::::::::: : : : : : 
P27115 MLKKQSAGLVLWGAILFVAWNALLLLFFWTRPVPSRLPSDNALDDDPASLTREVIRLAQD
      10    20    30    40    50    60


           70          80          90         100        110
/usr/t AEVELERQRGLLQQIGD--ALSSQRGRVPPTAAPPAPRVPVTPAPAIPILVIACDRSTV
       :::::::::::::: . : : : : : : : : : : : : : : : : : : : : : : : :
P27115 AEVELERQRGLLQQIREHHALWSQRWKVPTAAPPAPHVPVTPPPAPVIPILVIACDRSTV
      70     80     90     100     110     120


      120     130     140     150     160     170
/usr/t RRCLDKLLHYRPSAELFPPIIVSQDCGHEETAQAIAIASYGSAVTHIRQPDLSSIIVPPDHKR
       :::::::::::::::::::: : : : : : : : : : : : : : : : : : : : : : :
P27115 RRCLDKLLHYRPSAELFPPIIVSQDCGHEETAQVIAIASYGSAVTHIRQPDLSNIAVQPDKRK
      130     140     150     160     170     180


      180     190     200     210     220     230
/usr/t FQGYYKIARHYRWALGVFRQFRFPAAVVVEDDLEVAPDFFEYFRATYPPLLKADPSLWCV
       :::::::::::::::::::: : : : : ~~~~~~ : : : : : : : : : : : : : :
P27115 FQGYYKIARHYRWALGOIFHNFNYPAAVVVEDDLEVAPDFFEYFOATYPPLLKADPSLWCV
      190     200     210     220     230     240


      240     250     260     270     280     290
/usr/t SAWNDNGKEQMVDASRPPELLYRTDFFPGLGWLLLAE LWAELEPKWPKA FWDDWMRRPEQR
       :::::::::::::::::::: : : : : ~~~~~~ : : : : ~~~~~~ : : : : ~~~~~~
P27115 SAWNDNGKEQMVDSSKPPELLYRTDFFPGLGWLLLAE LWAELEPKWPKA FWDDWMRRPEQR
      250     260     270     280     290     300


      300     310     320     330     340     350
/usr/t QGRACIRPEISRTMTFG RKGVSHGQFFDQH LKF IKL NQQVF VHF TQL DLS YLQREAY DRDF
       ..::..::..::..::..::..::..::..::..::..::..::..::..::..::..::..
P27115 KGRACVRPEISRTMTFG RKGVSHGQFFDQH LKF IKL NQQVF VPFTQL DLS YLQ QEAY DRDF
      310     320     330     340     350     360


      360     370     380     390     400     410
/usr/t LARVYGAPQLQVEKV RTNDR KELGE VRVQYTGRDSFKAFAKALGVMD DLKS GVP RAGYRG
       :::::::::::::::::::: : : : : ~~~~~~ : : : : ~~~~~~ : : : : ~~~~~~
P27115 LARVYGAPQLQVEKV RTNDR KELGE VRVQYTGRDSFKAFAKALGVMD DLKS GVP RAGYRG
      370     380     390     400     410     420


      420     430     440
/usr/t IVTFQFRGRRVHL APPPTWEGY DPSWN
       :::: : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
P27115 IVTFLFRGRRVHL APQTWDGY DPSWT
      430     440

```

### Figure 6

## GnT1 Cys121Ser mutant

avipilviacdrstvrrsldkllyrpsaelfpiivsdcgheetaqaiasygsavthirqpdlssiaavppdhrkfqgyykiarhyrwa  
lgqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcvswndngkeqmvdasrpellyrtdffpglgwllaelwae  
lepkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqldlsylqreaydrdflarvyg  
apqlqvekvrtnrkelgevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpwn\*

Gcgggtgattcccatcctggtcatcgctgtgaccgcagcactgttcggcgctctctagacaagctgctgcattatcggccctcggctga  
gctcttcccatcatcggttagccaggactgcgggcacgaggagacggcccaggccatcgctcctacggcagcgcggtcacgcaca  
tccggcagcccagcctgagcagcattcggtgcccggaccaccgcaagttccagggtactacaagatcgcgcgccactaccg  
ctggcgctggggcaggctctccggcagtttcgcttccccggcgccgtggtggaggatgacctggaggtggccccggacttctt  
cgagtactttcgggccacctatccgctgctgaaggccgacccctccctgtggtgcgtctcggcctggaatgacaacggcaaggagca  
gatgggtggacgccagcaggcctgagctgctctaccgcaccgacttttccctggcctgggctggctgctgttgccgagctctgggt  
gagctggagcccaagtggccaaaggccttctgggacgactggatgcggcgccggagcagcggcagggcgggcctgcatacg  
ccctgagatctcaagaacgatgaccttggccgcaagggtgtgagccacgggcagttcttgaccagcacctcaagttatcaagctga  
accagcagttgtgacttcaccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgccgcgtctacggt  
gctccccagctgcaggtggagaaagtgaggaccaatgaccggaaggagctgggggaggtgcgggtgcagtatacgggcaggga  
cagcttcaaggcttccgcaaggctctgggtgtcatggatgaccttaagtcgggggttcgagagctggctaccgggtattgtcacctt  
ccagttccggggcccggtgtccacctggcgccccaccgacgtgggagggtatgatcctagctggaattag

Figure 7

## GnT1 Cys121Asp

avipilviacdrstvrrdlldkllhyrpsaelfpiivsdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwa  
lgqvfrqfrfpaavvveddleapdffeyfratypllkadpslwcv sawndngkeqmvdasrpellyrtdffpglgwllaelwae  
lepkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqldlsylqreaydrdflarvyg  
apqlqvekvrtnrkelgevrqvtytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlapptwegydpwn\*

Gcgggtgattcccatcctgggtcatgcctgtgaccgcagcactgttcggcgcgatctagacaagctgctgcattatcggccctcggtg  
agctcttccccatcatcgtagccaggactgcgggcacgaggagacggcccaggccatcgctcctacggcagcgcggtcacgcac  
atccggcagcccagcctgagcagcattgcggtgccgcccggaccaccgcaagttccagggtactacaagatcgcgcccactacc  
gctgggcgctgggccaggcttccggcagtttcgcttcccccgggcctggtggtggaggatgacctggaggtggccccggacttct  
tcgagtactttcgggccacctatccgctgctgaaggccgacccctccctgtggtgctctcggcctggaatgacaacggcaaggagc  
agatggtggacgccagcaggcctgagctgctctaccgcaccgacttttccctggcctgggctggctgctgttgccgagctctgggc  
tgagctggagcccaagtggccaaaggccttctgggacgactggatgcggcgccggagcagcgccaggggcgggcctgcatac  
gccctgagatctcaagaacgatgaccttggccgcaagggtgtgagccacgggcagttcttgaccagcacctcaagttatcaagctg  
aaccagcagtttgtcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgccgcgtctacgg  
tgctcccagctgcaggtggagaaagtgaggaccaatgaccggaaggagctgggggaggtcggggtgcagtatacgggcaggga  
cagcttcaaggcttgcgaaggctctgggtgtcatggatgaccttaagtcgggggticcgagagctggctaccgggtattgtcacctt  
ccagttccggggccgccgtgtccacctggcgccccaccgacgtgggagggtatgatcctagctggaattag

Figure 8



## GnT1 Cys121Thr

avipilviacdrstvrrtldkllhyrpsaelfpiivsdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwal  
gqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcvsawndngkeqmvdasrpellyrtdffpplglwlllaelwael  
epkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqlldsylqreaydrdflarvyg  
apqlqvekvrtndrkelgevrvtgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpawn\*

Gcgggtattcccatcctggatcgcctgtgaccgcagcactgttcggcgcaactctagacaagctgctgcattatcgccctcggctg  
agctcttcccatcatcgttagccaggactgcgggcacgaggagacggcccaggccatcgctcctacggcagcgcggtcacgcac  
atccggcagcccgacctgagcagcattgcgggtgccgccggaccacgcgaagtccagggtactacaagatcgcgccactacc  
gctgggcgctgggcccagggtcttcggcagttcgttccccgcggccgtgggtggaggatgacctggagggtggccccggacttct  
tcgagtactttcgggcccacctatccgtgctgaaggccgacccctccctgtgggtgcgtctcggcctggaatgacaacggcaaggagc  
agatgggtggacgccagcaggcctgagctgctctaccgcaccgacttttccctggcctgggctggctgctggtggccgagctctgggc  
tgagctggagcccaagtggccaaggccttctgggacgactggatgcggcgccggagcagcggcaggggcgggcctgcatac  
gccctgagatctcaagaacgatgaccttggccgcaagggtgtgagccacgggcagttctttgaccagcacctcaagttatcaagctg  
aaccagcagtttgtcacttcaccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttctcgccgcgtctacgg  
tgctccccagctgcaggtggagaaagtgaggaccaatgaccggaaggagctgggggaggtgcgggtgcagtatacgggcaggga  
cagcttcaaggcttctgccaaggctctgggtgtcatggatgaccttaagtcgggggtccgagagctggctaccggggtattgtcacctt  
ccagttcccgggccgctgtccacctggcgccccaccgacgtgggagggctatgacctaagctggaattag

Figure 9

## GnT1 Cys121Ala

avipilviacdrstvrraldkllhyrpsaelfpii vsqdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwa  
lgqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcv sawndngkeqmvdasrpellyrtdffpglgwlllaelwae  
lepkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqldlsylqreaydrdflarvyg  
apqlqvekvrtnrkelgevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpwn\*

Gcgggtgattcccatcctggtcatcgcctgtgaccgcagcactgttcggcgcgccctagacaagctgctgcattatcggccctcggctg  
agctcttcccatcatcgttagccaggactgcgggcacgaggagacggcccaggccatcgcctcctacggcagcgcggtcacgcac  
atccggcagcccgaacctgagcagcattgcgggtcccgccggaccaccgcaagttccagggtactacaagatcgcgcgccactacc  
gctggcgctgggccaggctctccggcagtttcgcttccccgcggccgtggtggaggatgacctggagggtggccccggacttct  
tcgagtactttcgggccacctatccgctgctgaaggccgaccttccctgtggtgctctcggcctggaatgacaacggcaaggagc  
agatggtggacgccagcaggcctgagctgctctaccgcaccgacttttccctggcctgggctggctgctgttgccgagctctgggc  
tgagctggagcccaagtggccaaaggccttctgggacgactggatgcggcggccggagcagcggcagggggcggcctgcatac  
gccctgagatcacaagaacgatgaccttggccgcaagggtgtgagccacgggcagttcttgaccagcacctcaagtttatcaagctg  
aaccagcagtttgtcacttcaccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctgcgccgctctacgg  
tgctccccagctgcaggtggagaaagtgaggaccaatgaccggaaggagctgggggaggtcgggtgcagtatacgggcaggga  
cagcttcaaggcttccgcaaggctctgggtgcatggatgaccttaagtcgggggttcgagagctggctaccgggtattgtcacctt  
ccagttcccgggccgccgtgtccacctggcgccccaccgacgtgggagggtatgatcctagctggaattag

Figure 10

## GnT1 Arg120Ala, Cys121H

avipilviacdrstvrahldklhlyrpsaelfpiivs qdcgheetaqaiasygsavthir qpdls siavppdhrkf qgyy kiarhyr w  
algqvfrqfrpaavvveddle vapdffeyfratyp llkadpslwcv sawndngkeqmv dasrpellyrtdffp glgwllaelwa  
elepkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftql dlsylqreaydrdflarvy  
gapqlqvekvrtndrkelgevr vqytgrdsfka fakalgvmddlksgvpragyr givtfqfp grrvhlappptwegydpswn\*

Gcgggtgattcccatcctgggtcatcgctgtgaccgcagcactgttcgggcccacctagacaagctgctgcattatcggccctcggctg  
agctcttccccatcatcggttagccaggactgcgggcacgaggagacggcccaggccatcgctcctacggcagcgcggtcacgcac  
atccggcagcccagacctgagcagcattgcggtgccgcggaccaccgcaagttccagggtactacaagatcgcgccactacc  
gctgggcgctgggcccaggcttccggcagtttcgcttccccgcggccgtggtggtggaggatgacctggagggtggccccggacttct  
tcgagtactttcgggccacctatccgctgctgaaggccgaccttccctgtggtgcgtctcgccctggaatgacaacggcaaggagc  
agatggtggacgccagcaggcctgagctgctctaccgcaccgacttttccctggcctgggctggctgctgttggccgagctctgggc  
tgagctggagcccaagtggccaaaggccttctgggacgactggatgcggcggccggagcagcggcaggggcgggcctgcatac  
gccctgagatctcaagaacgatgacctttggccgcaagggtgtgagccacgggcagttctttgaccagcacctcaagtttatcaagctg  
aaccagcagtttgtgacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttctcggccgctctacgg  
tgctccccagctgcaggtggagaaagtgaggaccaatgaccggaaggagctgggggaggtgcgggtgcagtatacgggcaggga  
cagcttcaaggctttcgccaaggctctgggtgtcatggatgaccttaagtcgggggttccgagagctggctaccggggtattgtcacctt  
ccagttccggggccgctgtccacctggcgccccaccgacgtgggagggctatgaccta gctggaattag

Figure 11

**Rat Liver ST3Gal III amino acid sequence:**

MGLLVFVRNLLLALCLFLVLGFLYSAWKLHLLQWEDSNLILSLDSAGQTLGTEYDRL  
GFLKLDSKLPaelATKYANFSEGACKPGYASAMMTAIFPRFSKPAPMFLDDSF~~R~~KW  
ARIREFVPPFGIKGQDNLIKAILSVTKEYRLTPALDSLHCRRCTIVGNGGVLAN~~K~~SLGS  
RID~~D~~YDIVIRLNSAPVKGF~~E~~KDVGSKTTLRITYPEGAMQRPEQYERDSL~~F~~VLAGFKW  
QDFKWLKYIVYKERV~~S~~ASDGF~~W~~KSVATRV~~P~~KEPPEIRILNPYFIQEA~~A~~FTLIGLPFNN  
GLMGRGNIPTLGSA~~V~~TMALDGCDEVAVAGFGYDMNTPNAPLHYYETVRMAA~~I~~KE  
SWTHNIQREKEFLRKL~~V~~KARVITDLSSGI

**Figure 12**

# **Full length UDP-N-acetylgalactosaminyltransferase 2 (GalNAcT2) nucleic acid and amino acid sequences**

## **Amino acid sequence**

Met	Arg	Arg	Arg	Ser	Arg	Met	Leu	Leu	Cys	Phe	Ala	Phe	Leu	Trp	Val	1	5	10	15
Leu	Gly	Ile	Ala	Tyr	Tyr	Met	Tyr	Ser	Gly	Gly	Gly	Ser	Ala	Leu	Ala	20	25	30	
Gly	Gly	Ala	Gly	Gly	Gly	Ala	Gly	Arg	Lys	Glu	Asp	Trp	Asn	Glu	Ile	35	40	45	
Asp	Pro	Ile	Lys	Lys	Lys	Asp	Leu	His	His	Ser	Asn	Gly	Glu	Glu	Lys	50	55	60	
Ala	Gln	Ser	Met	Glu	Thr	Leu	Pro	Pro	Gly	Lys	Val	Arg	Trp	Pro	Asp	65	70	75	80
Phe	Asn	Gln	Glu	Ala	Tyr	Val	Gly	Gly	Thr	Met	Val	Arg	Ser	Gly	Gln	85	90	95	
Asp	Pro	Tyr	Ala	Arg	Asn	Lys	Phe	Asn	Gln	Val	Glu	Ser	Asp	Lys	Leu	100	105	110	
Arg	Met	Asp	Arg	Ala	Ile	Pro	Asp	Thr	Arg	His	Asp	Gln	Cys	Gln	Arg	115	120	125	
Lys	Gln	Trp	Arg	Val	Asp	Leu	Pro	Ala	Thr	Ser	Val	Val	Ile	Thr	Phe	130	135	140	
His	Asn	Glu	Ala	Arg	Ser	Ala	Leu	Leu	Arg	Thr	Val	Val	Ser	Val	Leu	145	150	155	160
Lys	Lys	Ser	Pro	Pro	His	Leu	Ile	Lys	Glu	Ile	Ile	Leu	Val	Asp	Asp	165	170	175	
Tyr	Ser	Asn	Asp	Pro	Glu	Asp	Gly	Ala	Leu	Leu	Gly	Lys	Ile	Glu	Lys	180	185	190	
Val	Arg	Val	Leu	Arg	Asn	Asp	Arg	Arg	Glu	Gly	Leu	Met	Arg	Ser	Arg	195	200	205	
Val	Arg	Gly	Ala	Asp	Ala	Ala	Gln	Ala	Lys	Val	Leu	Thr	Phe	Leu	Asp	210	215	220	
Ser	His	Cys	Glu	Cys	Asn	Glu	His	Trp	Leu	Glu	Pro	Leu	Leu	Glu	Arg	225	230	235	240
Val	Ala	Glu	Asp	Arg	Thr	Arg	Val	Val	Ser	Pro	Ile	Ile	Asp	Val	Ile	245	250	255	
Asn	Met	Asp	Asn	Phe	Gln	Tyr	Val	Gly	Ala	Ser	Ala	Asp	Leu	Lys	Gly	260	265	270	
Gly	Phe	Asp	Trp	Asn	Leu	Val	Phe	Lys	Trp	Asp	Tyr	Met	Thr	Pro	Glu	275	280	285	

**FIG. 13A (1/2)**

**FIG. 13A (2/2)**

**Nucleic acid sequence**

atgcgggcggc gctcgcggat gctgctctgc ttcgccttcc tgtgggtgct gggcatcgcc	60
tactacatgt actcgggggg cggctctgcg ctggccgggg gcgcggggcg cggcgccggc	120
aggaaggagg actggaatga aattgacccc attaaaaaga aagaccttca tcacagcaat	180
ggagaagaga aagcacaaag catggagacc ctccctccag ggaaagtacg gtggccagac	240
tttaaccagg aagcttatgt tggagggacg atggctcgct ccgggcagga cccttacgcc	300
cgcaacaagt tcaaccagggt ggagagtgat aagcttcgaa tggacagagc catccctgac	360
acccggcatg accagtgtca gcggaagcag tggcgggtgg atctgccggc caccagcgtg	420
gtgatcacgt ttcacaatga agccaggtcg gccctactca ggaccgtggt cagcgtgctt	480
aagaaaagcc cgccccatct cataaaagaa atcatcttgg tggatgacta cagcaatgat	540
cctgaggacg gggctctctt ggggaaaatt gagaaagtgc gagttcttag aaatgatcga	600
cgagaaggcc tcatgcgctc acgggttcgg ggggccgatg ctgcccaagc caaggtcctg	660
accttccttg acagtcactg cgagtgtaat gagcactggc tggagccctt cctggaaagg	720
gtggcggagg acaggactcg ggttggtgtca cccatcatcg atgtcattaa tatggacaac	780
tttcagtatg tgggggcacg tgctgacttg aagggcgggt ttgattggaa cttggtattc	840
aagtgggatt acatgacgcc tgagcagaga aggtcccggc aggggaaccc agtcgcccct	900
ataaaaaccc ccatgattgc tgggtgggctg tttgtgatgg ataagttcta ttttgaagaa	960
ctggggaagt acgacatgat gatggatgtg tggggaggag agaacctaga gatctcgttc	1020
cgcgtgtggc agtgtggtgg cagcctggag atcatccgt gcagccgtgt gggacacgtg	1080
ttccggaagc agcacccta cacgttcccg ggtggcagtg gcactgtctt tgcccgaac	1140
acccgccggg cagcagaggt ctggatggat gaatacaaaa atttctatta tgcagcagtg	1200
ccttctgcta gaaacgttcc ttatggaaat attcagagca gattggagct taggaagaaa	1260
ctcagctgca agcctttcaa atggtacott gaaaatgtct atccagagtt aagggttcca	1320
gaccatcagg atatagcttt tggggccttg cagcagggaa ctaactgcct cgacactttg	1380
ggacactttg ctgatggtgt ggttggagtt tatgaatgtc acaatgctgg gggaaaccag	1440
gaatgggcct tgacgaagga gaagtgggtg aagcacatgg atttgtgcct tactgtggtg	1500
gaccgggcac cgggctctct tataaagctg cagggtgcc gagaaaatga cagcagacag	1560
aaatgggaac agatcgaggg caactccaag ctgaggcacg tgggcagcaa cctgtgcctg	1620
gacagtgcga cggccaagag cgggggccta agcgtggagg tgtgtggccc ggccctttcg	1680
cagcagtgga agttcacgct caacctgcag cag	1713

**Figure 13B**

# **Δ51 UDP-N-acetylgalactosaminyltransferase 2, GalNAcT2, nucleic acid and amino acid sequences**

## **Amino acid sequence**

Lys Lys Lys Asp Leu His His Ser Asn Gly Glu Glu Lys Ala Gln Ser  
 1 5 10 15  
 Met Glu Thr Leu Pro Pro Gly Lys Val Arg Trp Pro Asp Phe Asn Gln  
 20 25 30  
 Glu Ala Tyr Val Gly Gly Thr Met Val Arg Ser Gly Gln Asp Pro Tyr  
 35 40 45  
 Ala Arg Asn Lys Phe Asn Gln Val Glu Ser Asp Lys Leu Arg Met Asp  
 50 55 60  
 Arg Ala Ile Pro Asp Thr Arg His Asp Gln Cys Gln Arg Lys Gln Trp  
 65 70 75 80  
 Arg Val Asp Leu Pro Ala Thr Ser Val Val Ile Thr Phe His Asn Glu  
 85 90 95  
 Ala Arg Ser Ala Leu Leu Arg Thr Val Val Ser Val Leu Lys Lys Ser  
 100 105 110  
 Pro Pro His Leu Ile Lys Glu Ile Ile Leu Val Asp Asp Tyr Ser Asn  
 115 120 125  
 Asp Pro Glu Asp Gly Ala Leu Leu Gly Lys Ile Glu Lys Val Arg Val  
 130 135 140  
 Leu Arg Asn Asp Arg Arg Glu Gly Leu Met Arg Ser Arg Val Arg Gly  
 145 150 155 160  
 Ala Asp Ala Ala Gln Ala Lys Val Leu Thr Phe Leu Asp Ser His Cys  
 165 170 175  
 Glu Cys Asn Glu His Trp Leu Glu Pro Leu Leu Glu Arg Val Ala Glu  
 180 185 190  
 Asp Arg Thr Arg Val Val Ser Pro Ile Ile Asp Val Ile Asn Met Asp  
 195 200 205  
 Asn Phe Gln Tyr Val Gly Ala Ser Ala Asp Leu Lys Gly Gly Phe Asp  
 210 215 220  
 Trp Asn Leu Val Phe Lys Trp Asp Tyr Met Thr Pro Glu Gln Arg Arg  
 225 230 235 240  
 Ser Arg Gln Gly Asn Pro Val Ala Pro Ile Lys Thr Pro Met Ile Ala  
 245 250 255  
 Gly Gly Leu Phe Val Met Asp Lys Phe Tyr Phe Glu Glu Leu Gly Lys  
 260 265 270  
 Tyr Asp Met Met Met Asp Val Trp Gly Gly Glu Asn Leu Glu Ile Ser  
 275 280 285  
 Phe Arg Val Trp Gln Cys Gly Gly Ser Leu Glu Ile Ile Pro Cys Ser

**FIG. 14A (1/2)**



290		295		300
Arg Val Gly His Val	Phe Arg Lys Gln His	Pro Tyr Thr Phe Pro Gly		
305	310	315		320
Gly Ser Gly Thr Val	Phe Ala Arg Asn Thr	Arg Arg Ala Ala Glu Val		
	325	330		335
Trp Met Asp Glu Tyr Lys	Asn Phe Tyr Tyr Ala Ala Val	Pro Ser Ala		
	340	345		350
Arg Asn Val Pro Tyr Gly	Asn Ile Gln Ser Arg Leu Glu	Leu Arg Lys		
	355	360		365
Lys Leu Ser Cys Lys Pro	Phe Lys Trp Tyr Leu Glu	Asn Val Tyr Pro		
	370	375		380
Glu Leu Arg Val Pro Asp	His Gln Asp Ile Ala Phe Gly Ala Leu Gln			
385	390	395		400
Gln Gly Thr Asn Cys Leu	Asp Thr Leu Gly His Phe Ala Asp Gly Val			
	405	410		415
Val Gly Val Tyr Glu Cys	His Asn Ala Gly Gly Asn Gln Glu Trp Ala			
	420	425		430
Leu Thr Lys Glu Lys Ser	Val Lys His Met Asp Leu Cys Leu Thr Val			
	435	440		445
Val Asp Arg Ala Pro Gly	Ser Leu Ile Lys Leu Gln Gly Cys Arg Glu			
	450	455		460
Asn Asp Ser Arg Gln Lys	Trp Glu Gln Ile Glu Gly Asn Ser Lys Leu			
465	470	475		480
Arg His Val Gly Ser Asn	Leu Cys Leu Asp Ser Arg Thr Ala Lys Ser			
	485	490		495
Gly Gly Leu Ser Val Glu	Val Cys Gly Pro Ala Leu Ser Gln Gln Trp			
	500	505		510
Lys Phe Thr Leu Asn Leu	Gln Gln			
	515	520		

FIG. 14A (2/2)

**Nucleic acid sequence**

aaaaagaaaag	acotcatca	cagcaatgga	gaagagaaaag	cacaaagcat	ggagaccctc	60
cctccaggga	aagtacggtg	gccagacttt	aaccaggaag	cttatgttgg	agggacgatg	120
gtccgctccg	ggcaggaccc	ttacgcccgc	aacaagttca	accaggtgga	gagtgataag	180
cttcgaatgg	acagagccat	ccctgacacc	cggcatgacc	agtgtcagcg	gaagcagtgg	240
cgggtggatc	tgccggccac	cagcgtggtg	atcacgtttc	acaatgaagc	caggtcggcc	300
ctactcagga	ccgtggtcag	cgtgcttaag	aaaagcccgc	cccatctcat	aaaagaaatc	360
atcttggtgg	atgactacag	caatgatcct	gaggacgggg	ctctcttggg	gaaaattgag	420
aaagtgcgag	ttottagaaa	tgatcgacga	gaaggcctca	tgcgctcacg	ggttcggggg	480
gccgatgctg	cccaagccaa	ggtcctgacc	ttcctggaca	gtcactgcga	gtgtaatgag	540
cactggctgg	agccctcct	ggaaaggggtg	gcgagggaca	ggactcgggt	tgtgtcacc	600
atcatcgatg	tcattaatat	ggacaacttt	cagtatgtgg	gggcatctgc	tgacttgaag	660
ggcggttttg	attggaactt	ggtattcaag	tgggattaca	tgacgcctga	gcagagaagg	720
tcccggcagg	ggaacccagt	cgcccctata	aaaaccccca	tgattgctgg	tgggctgttt	780
gtgatggata	agttctat	tgaagaactg	gggaagtacg	acatgatgat	ggatgtgtgg	840
ggaggagaga	acctagagat	ctcgttcgcg	gtgtggcagt	gtggtggcag	cctggagatc	900
atcccgtgca	gccgtgtggg	acacgtgttc	cgggaagcagc	acccctacac	gttcccggtt	960
ggcagtggca	ctgtctttgc	ccgaaacacc	cgccgggcag	cagaggtctg	gatggatgaa	1020
tacaaaaatt	tctattatgc	agcagtgcct	tctgctagaa	acgttcctta	tggaaatatt	1080
cagagcagat	tggagcttag	gaagaaactc	agctgcaagc	ctttcaa	gtaccttgaa	1140
aatgtctatc	cagagttaag	ggttcagac	catcaggata	tagcttttgg	ggccttgcag	1200
cagggaaacta	actgcctoga	cactttggga	cactttgctg	atggtgtggt	tggagtttat	1260
gaatgtcaca	atgctggggg	aaaccaggaa	tgggccttga	cgaaggagaa	gtcgggtgaag	1320
cacatggatt	tgtgccttac	tgtggtggac	cgggcaccgg	gctctcttat	aaagctgcag	1380
ggctgccgag	aaaatgacag	cagacagaaa	tgggaacaga	tcgagggcaa	ctccaagctg	1440
aggcacgtgg	gcagcaacct	gtgcctggac	agtcgcacgg	ccaagagcgg	gggcctaagc	1500
gtggaggtgt	gtggcccggc	cctttcgcag	cagtgggaagt	tcacgctcaa	cctgcagcag	1560

**Figure 14B**

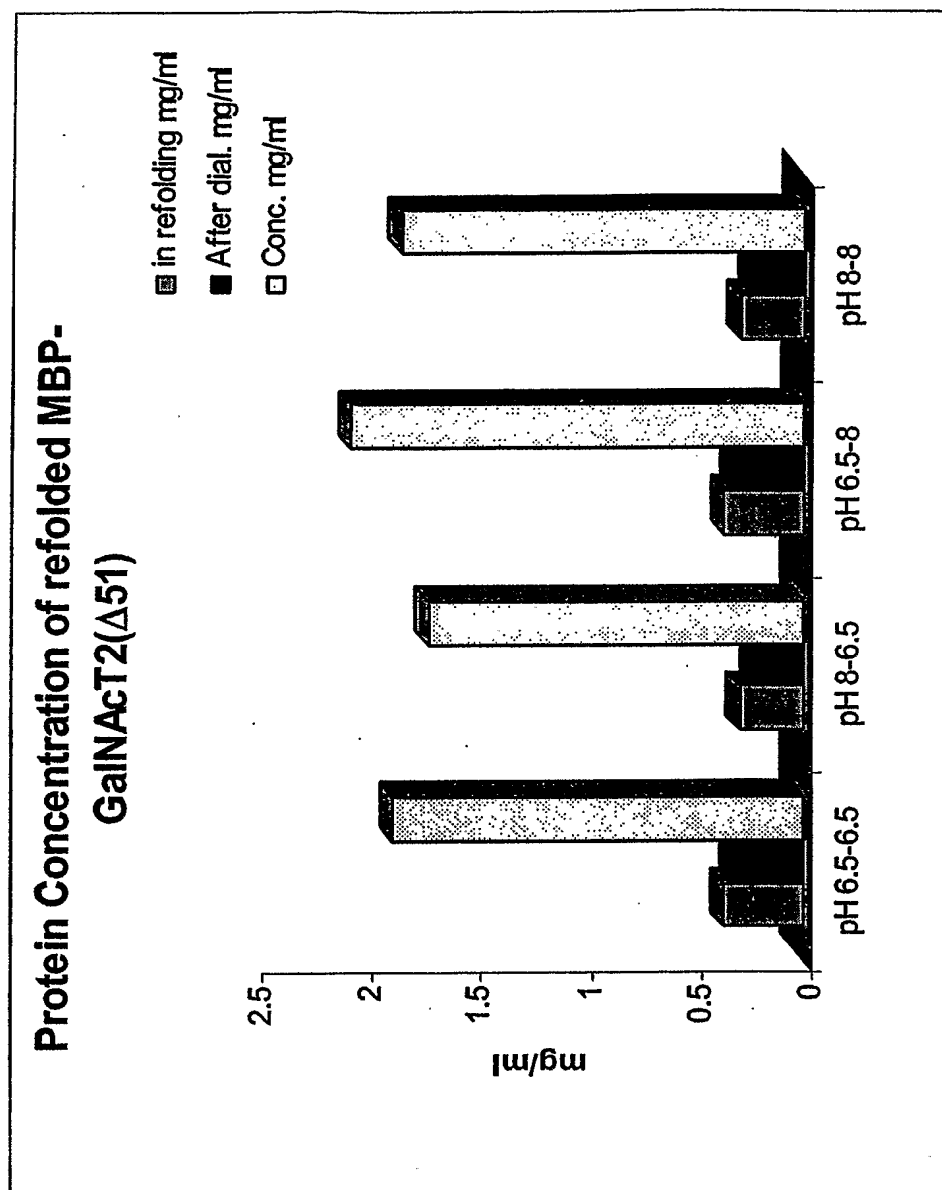


Figure 15

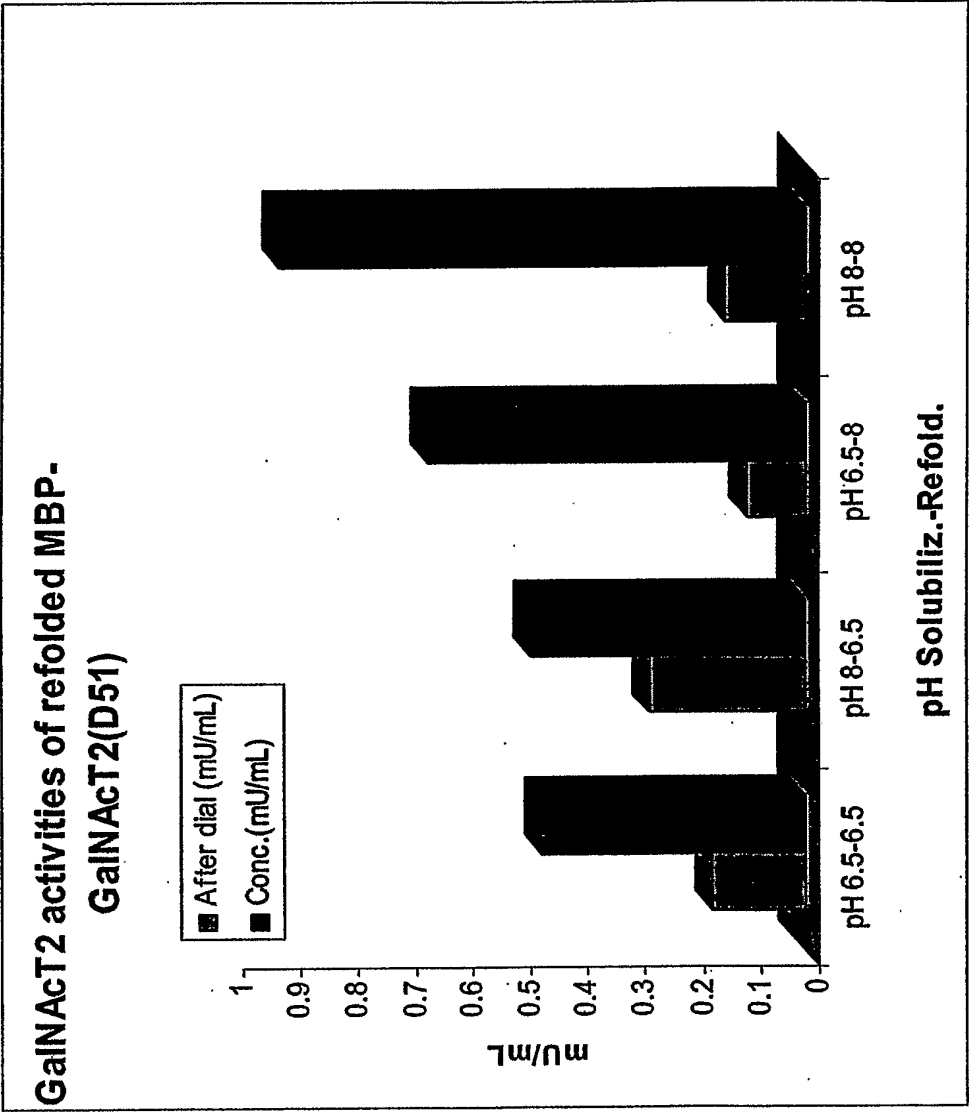
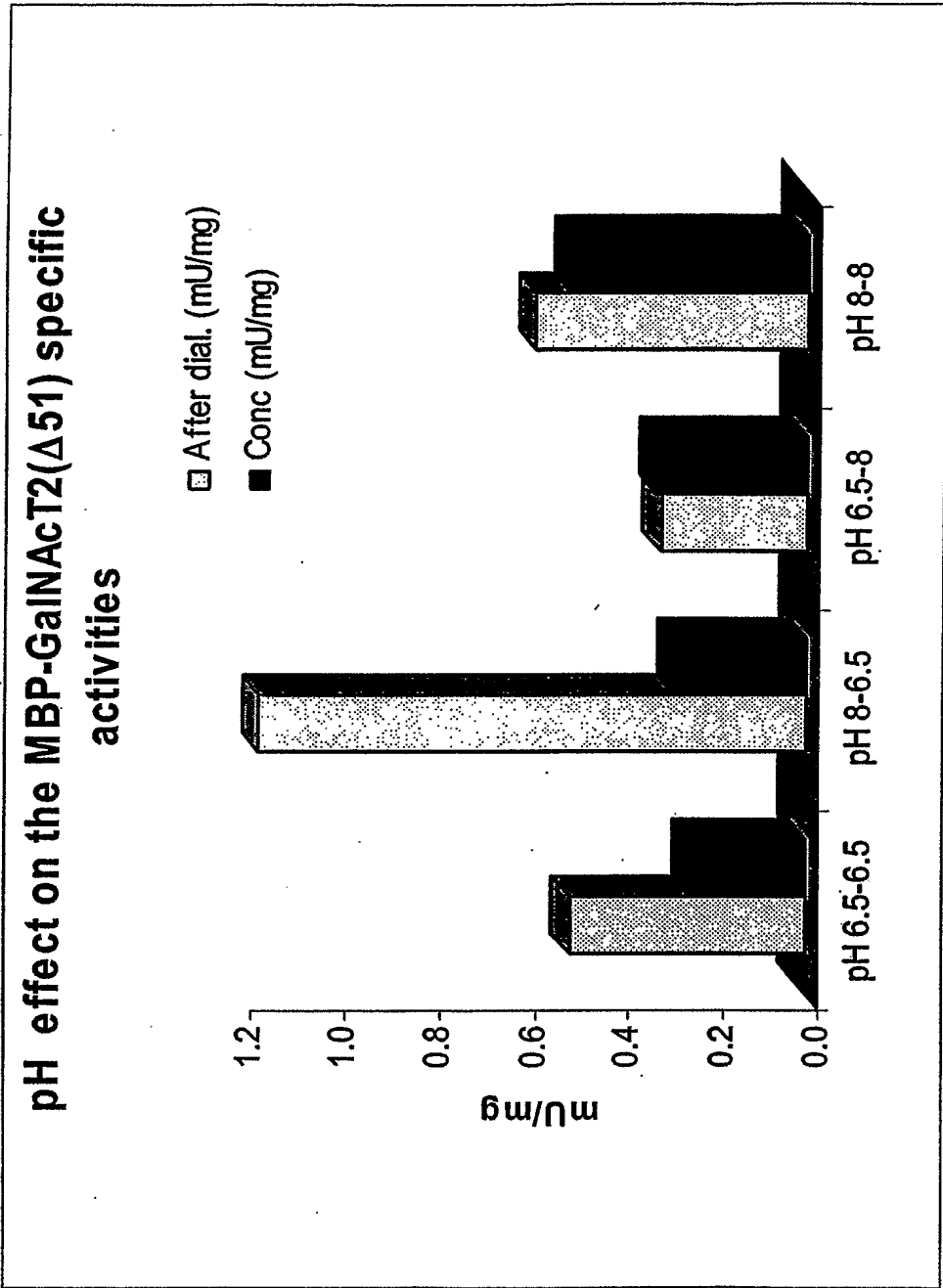


Figure 16



pH Solubiliz. -- refold)

Figure 17

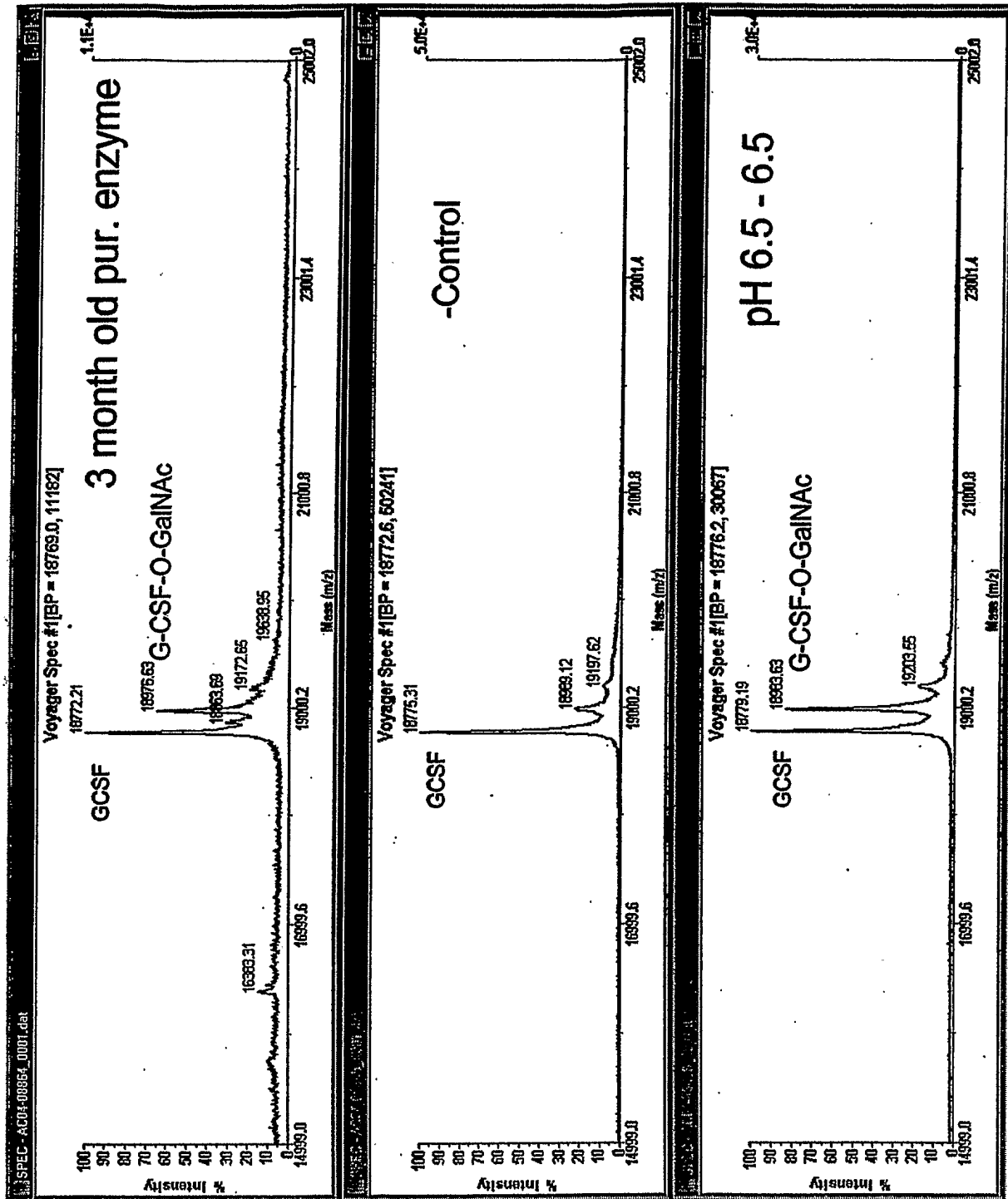


Figure 18A

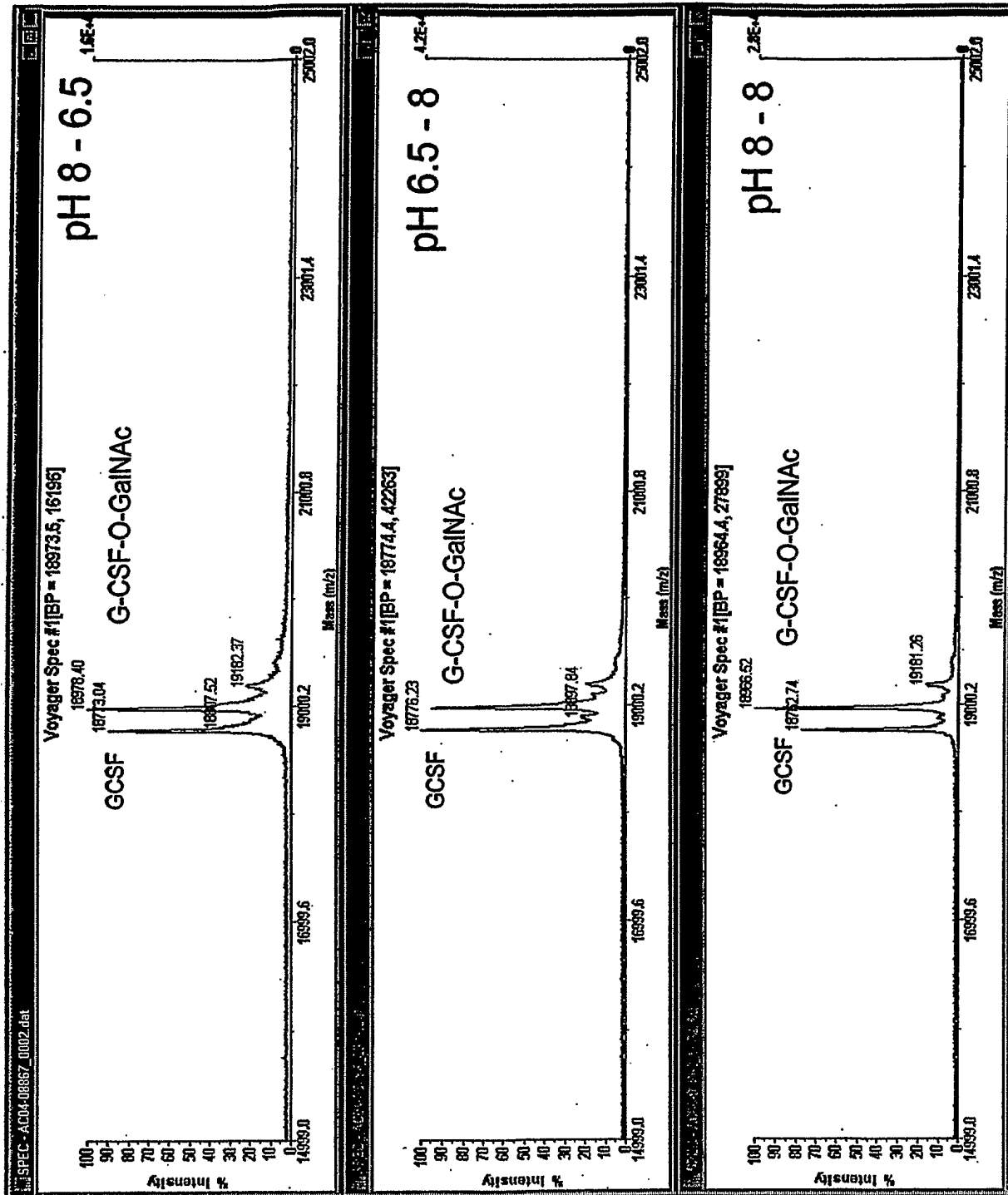
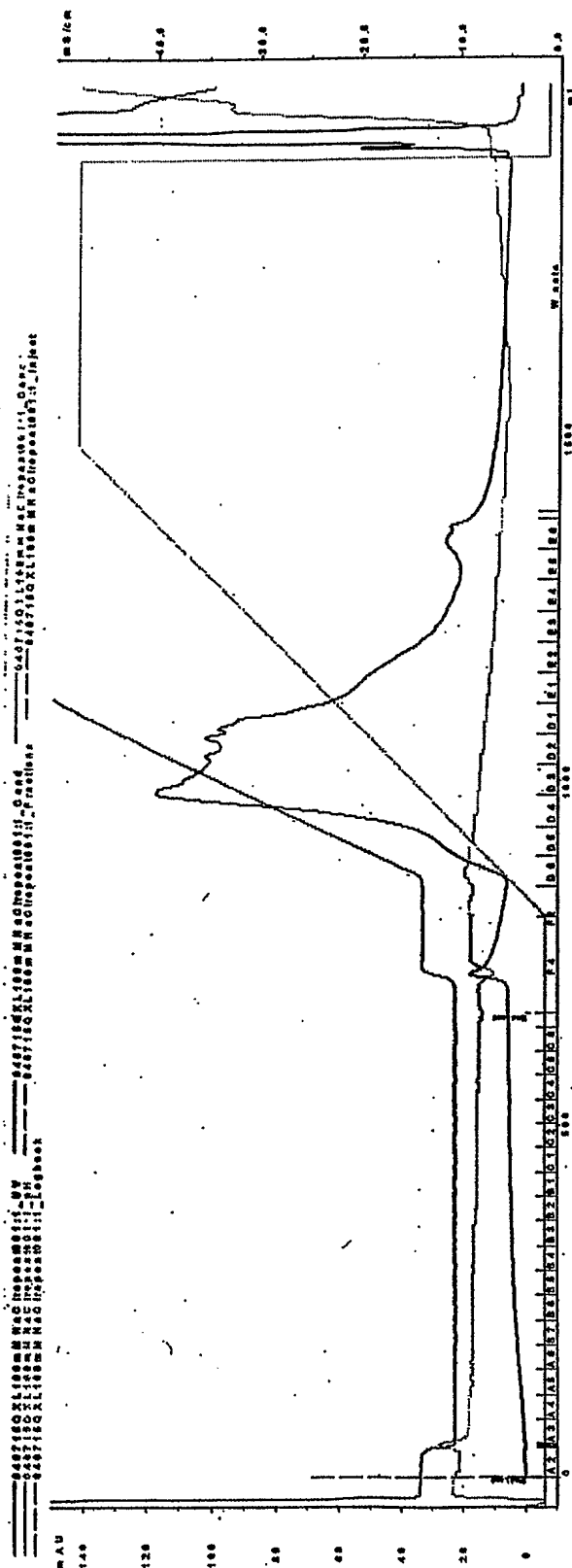


Figure 18B



- |            |             |
|------------|-------------|
| 1. Load    | 11. Wash    |
| 2. A2/3    | 12. D6      |
| 3. A4/5    | 13. D5      |
| 4. A6/B7   | 14. D4      |
| 5. B6/5    | 15. D3/2    |
| 6. B4/3    | 16. D1/E1/2 |
| 7. B2/1    | 17. E6      |
| 8. C1/2    | 18. Load    |
| 9. C3/4    |             |
| 10. C5/6/7 |             |

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18



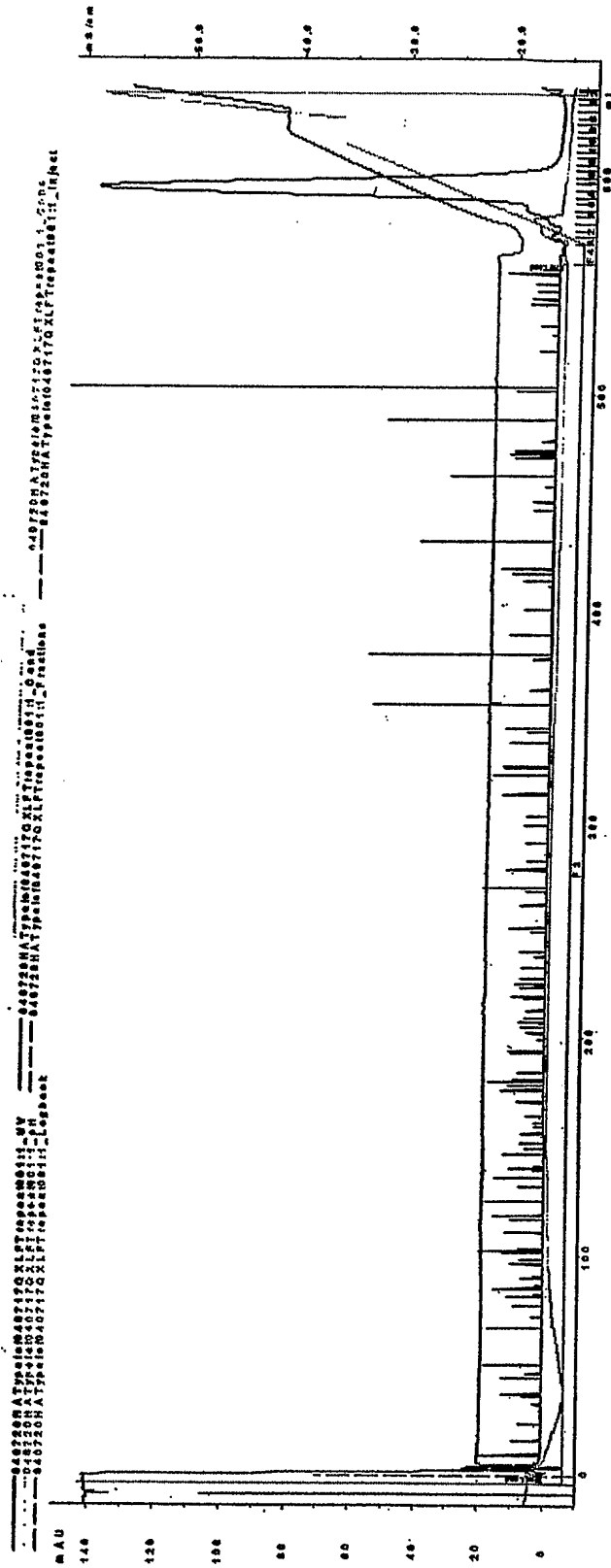
pooled

FIG. 19



	Volume (mL)	Activity (U/L)	A280
Load	890	1.5	0.110
FT <sub>A4-C4</sub>	670	9.2	NA
FT <sub>C5-C7</sub>	120	1.0	0
Wash	138	3.6	0.100
D6	45	4.5	0
D5	45	2.4	0.026
D4	45	2.0	0.108
D3/2	90	1.1	0.179
E6		0.0	0.017

FIG. 20



L FT WA5/6 A7 A8 A9 A10 A11

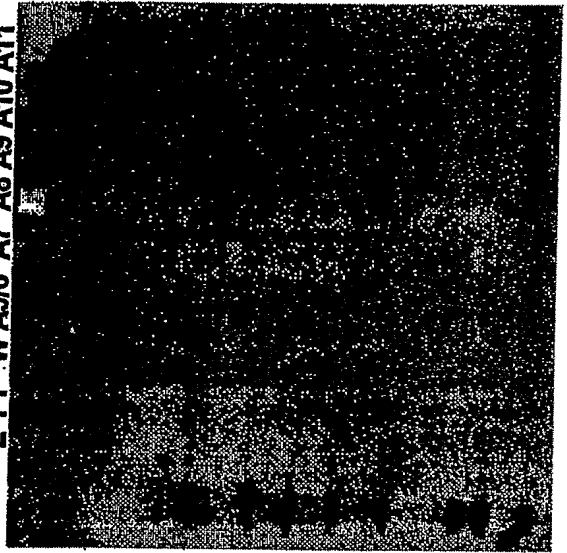


FIG. 21

Pooled A4-C4 and adjusted pH to 7.0 using 1mM HCl 670mL  
load pH 7.0 16mS/cm

	Volume (mL)	Activity (U/L) pre- dialysis	Activity (U/L) post- dialysis	A280	A280/ 1.51 (mg/mL)	Activity (U)	Mass (mg)	Specific Activity (U/mg)
Load	670	9.2	NA	NA				
FT	670	0.0	NA	0.122				
Wash	9	2.9	NA	-0.013				
A5/6	6	1.1	NA	-0.005				
A7	3	0.1	19.3 (13mL)	0.180	0.119	0.25	1.55	0.16
A8	3	1.3						
A9	3	4.6						
A10	3	2.4						
A11	3	0.4						

FIG. 22

# COMPARISON of MBP with MBP-SBD<sub>39</sub> tag in ST3Gal3 in Cyclodextrin column purification

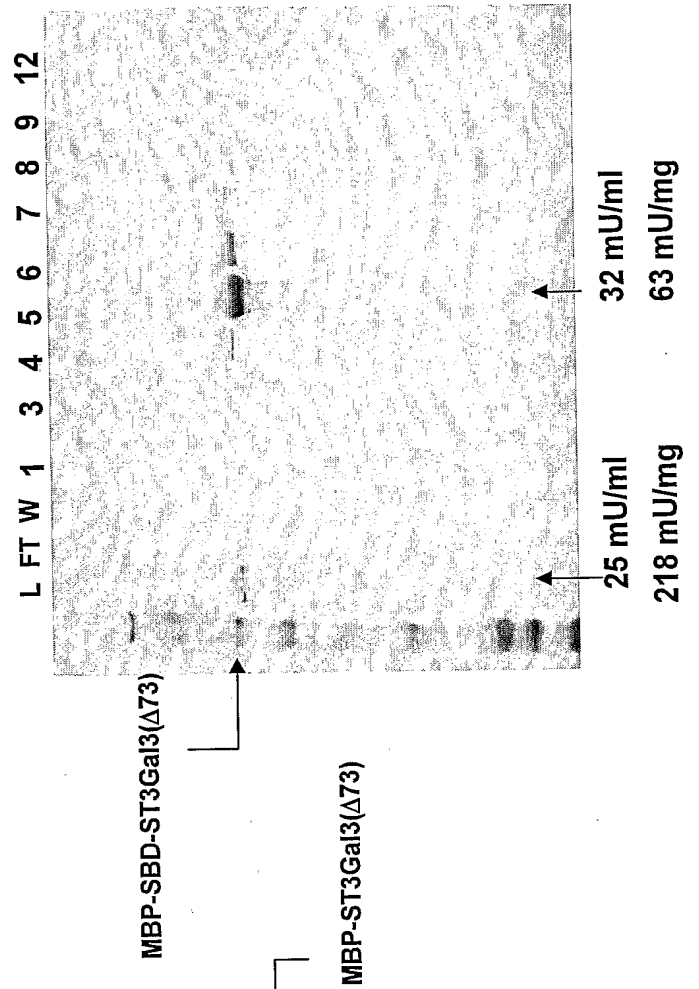
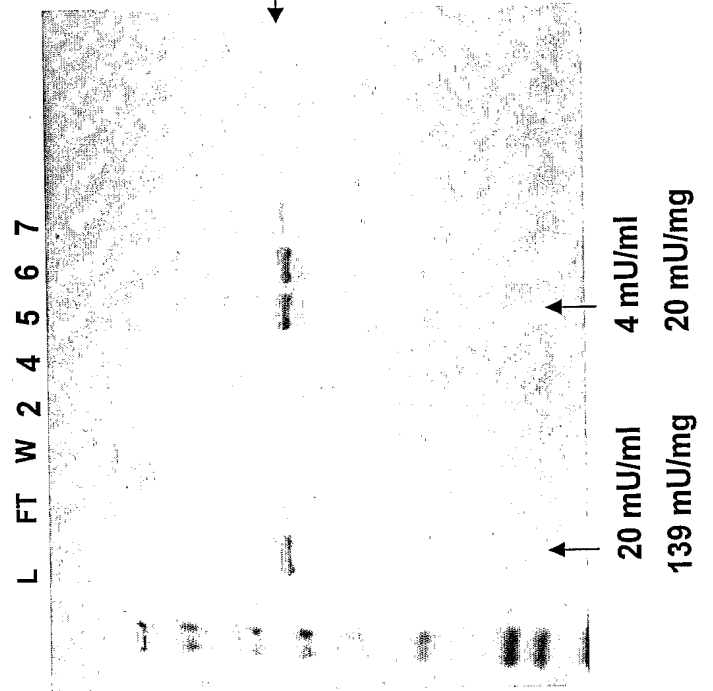
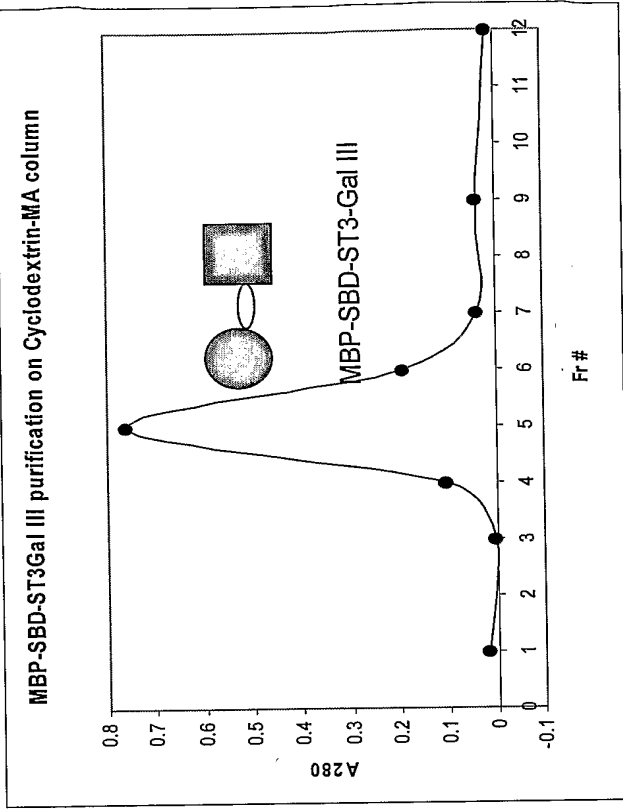
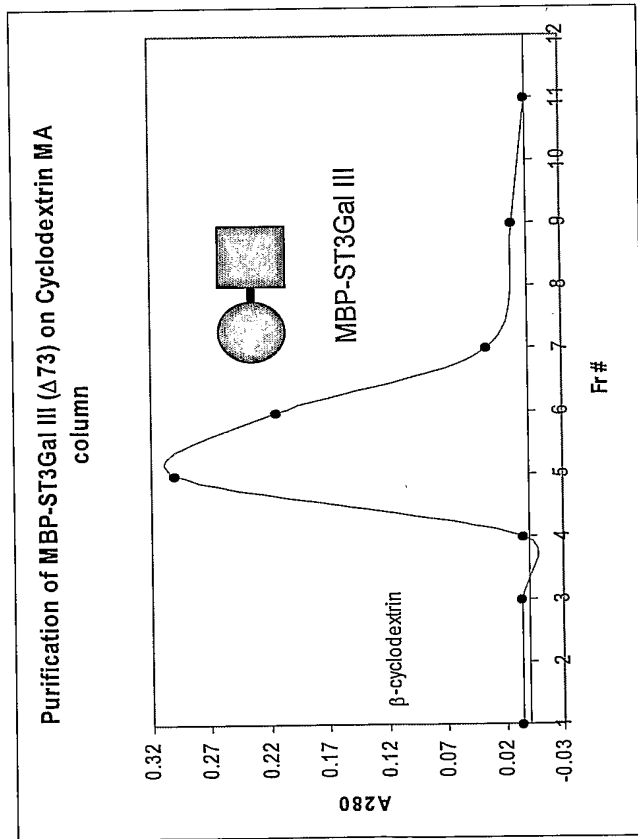


Figure 24

## a) MBP-pST3Gal1 fusion protein

MKIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFFWAHDRF  
GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD  
KELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDNAGAKAGLTFLVDLIKNGH  
MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP  
NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAF  
WYAVRTAVINAASGRQTVDEALKDAQTNSNNNNNNNNNNNNNLGIEGRISEFGSELSSENFKKLMKYPYR  
PCTCTRCIEEQRVSAWFDERFNRSMQPLLAKNAHLEEDTYKWWLRLQREKQPNNLNDTIRELFQVVP  
GNVDPLLEKRLVSCRRCAVVGNSGNLKESYYGPQIDSHDFVLRMNKAPTEGFEADVGSKTTHHFVYPE  
SFRELAQEVSMILVPFKTTDLEWVISATTTGRISHTYVPVPAKIKVKEKILYHPAFIKYVFDRWLQGH  
GRYPSTGILSVIFSLHICDEVDLYGFGADSKGNWHHYWENNPSAGAFRKTGVHDGDFESNVTTILASIN  
KIRIFKGR

## b) MBPSBD-pST3Gal1 fusion protein

MKIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFFWAHDRF  
GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD  
KELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDNAGAKAGLTFLVDLIKNGH  
MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP  
NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIQMSAF  
WYAVRTAVINAASGRQTVDEALKDAQTNSNNNNNNNNNNNNNLGIEGRISEFGSIVATGGTTTTATPTG  
SGSVTSTSKTTATASKTSTSTSTSTCTTPTAVAVTFDLTATTTYGENIYLVGSIQLGDWETSDGIALSAD  
KYTSSDPLWYVTVTLPAGESEYKFIRESDDSVESDPNREYTPVQACGTSTATVTDWTRGSELSSENF  
FKKLMKYPYRPTCTRCIEEQRVSAWFDERFNRSMQPLLAKNAHLEEDTYKWWLRLQREKQPNNLN  
DTIRELFQVVPGNVDPLLEKRLVSCRRCAVVGNSGNLKESYYGPQIDSHDFVLRMNKAPTEGFEADV  
SKTTHHFVYPESFRELAQEVSMILVPFKTTDLEWVISATTTGRISHTYVPVPAKIKVKEKILYHPAFIK  
YVFDRWLQGHGRYPSTGILSVIFSLHICDEVDLYGFGADSKGNWHHYWENNPSAGAFRKTGVHDGDF  
ESNVTTILASINKIRIFKGR

Figure 25

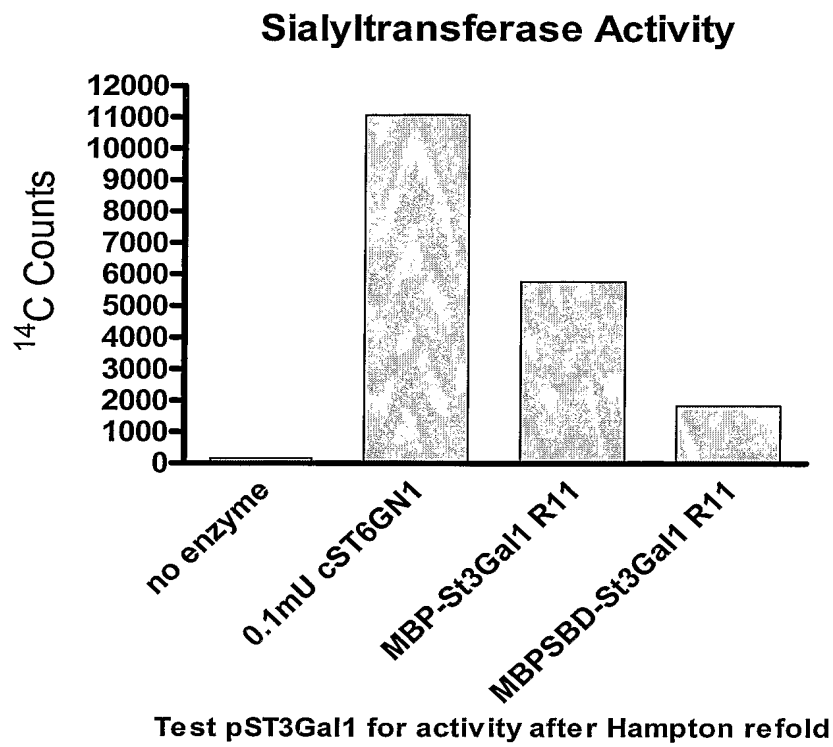


Figure 26

A.

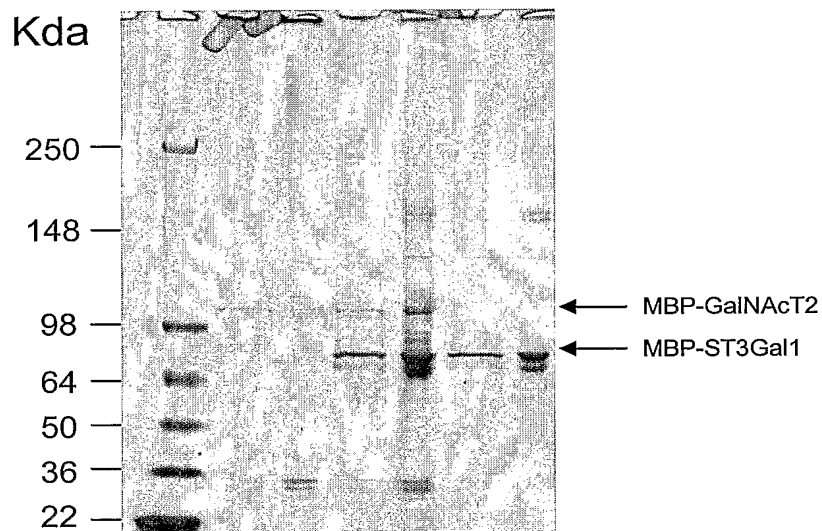
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KELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKXKH  
MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGLSAGINAASP  
NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYYEELAKDPRIAATMENAQKGEIMPNIPQMSAF  
WYAVRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNNNLGIEGRISEFGSSEHLDKVPRTPGAL  
STRKTPMATGAVPAKKKVQATKSPASSPHPTTRRRQRLKASEFKSEPRWDFEEEEEYSLDMSSLQT  
NCSASVKIKASKSPWLQNIFLPNITLFLDSGRFTQSEWNRLEHFAPPFGFMELNQSLVQKVVTFRP  
PVRQQQLLLASLPTGYSKCITCAVVGNNGILNDSRVGREIDSHDYVFRLSGAVIKGYEQDVGTRT  
SFYGFATAFSLTQSILILGRRGFQHVPLGKDVRYLHFLEGRNYEWLEAMFLNQTLAKTHLSWFR  
HRPQEAFRNALDLDRYLLHPDFLRYMKNRFLRSKTLDTAXWRIYRPTTGALLLLTALHLCDKV  
SAYGFITEGHERFSDHYDYTSWKRLIFYINHDFRLERMVWKRLHDEGIWLYQRPQSDKAKN

B.

MKIEEGKLVWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIFWAHDFR  
GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD  
KELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIKXKH  
MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGLSAGINAASP  
NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYYEELAKDPRIAATMENAQKGEIMPNIPQMSAF  
WYAVRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNNNLGIEGRISEFGSKEPQTKPSRHQRTE  
NIKERSLQSLAKPKSQAPTRARRTTIYAEPVPENNALNTQTQPKAHTTGDRGKEANQAPPEEQDK  
VPHTAQRAAWKSPEKEKTMVNTLSPRGQDAGMASGRTEAQSWKSQDTKTTQGNNGGQTRKLT  
SRTVSEKHQGAATTAKTLIPKSQHRMLAPTGAVSTRTRQKGVTTAVIPPKEKKPQATPPAPFQ  
SPTTQRNQRLKAANFKSEPRWDFEEKYSFEIGGLQTTCPDSVKIKASKSLWLQKFLPNLTLFLDS  
RHFNQSEWDRLEHFAPPFGFMELNYSLVQKVVTFRPPVPPQQQLLLASLPAGSLRCITCAVVGN  
GILNNSHMGQEIDSHDYVFRLSGALIKGYEQDVGTRTSFYGFATAFSLTQSLILGNRGFKNVPLGK  
DVRYLHFLEGRDYEWLEALLMNQTVMSKNLFWFRHRPQEAFFREALHMDRYLLHPDFLRYM  
KNRFLRSKTLDDGAHWRIYRPTTGALLLLTALQLCDQVSAYGFITEGHERFSDHYDYTSWKRLIFY  
INHDFKLEREVWKRLHDEGIWLYQRPQPGTAKAKN

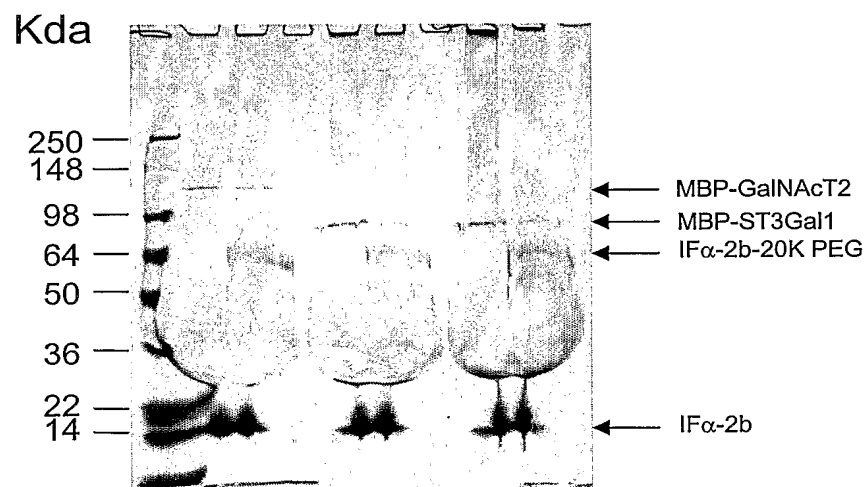
Figure 27

A.



Lanes: 1) See Blue Plus 2  
 2) MBP-GalNAcT2  
 3) MBP-GalNAcT2 (conc)  
 4) Co-refold  
 5) Co-refold (conc)  
 6) MBP-ST3Gal1  
 7) MBP-ST3Gal1 (conc)

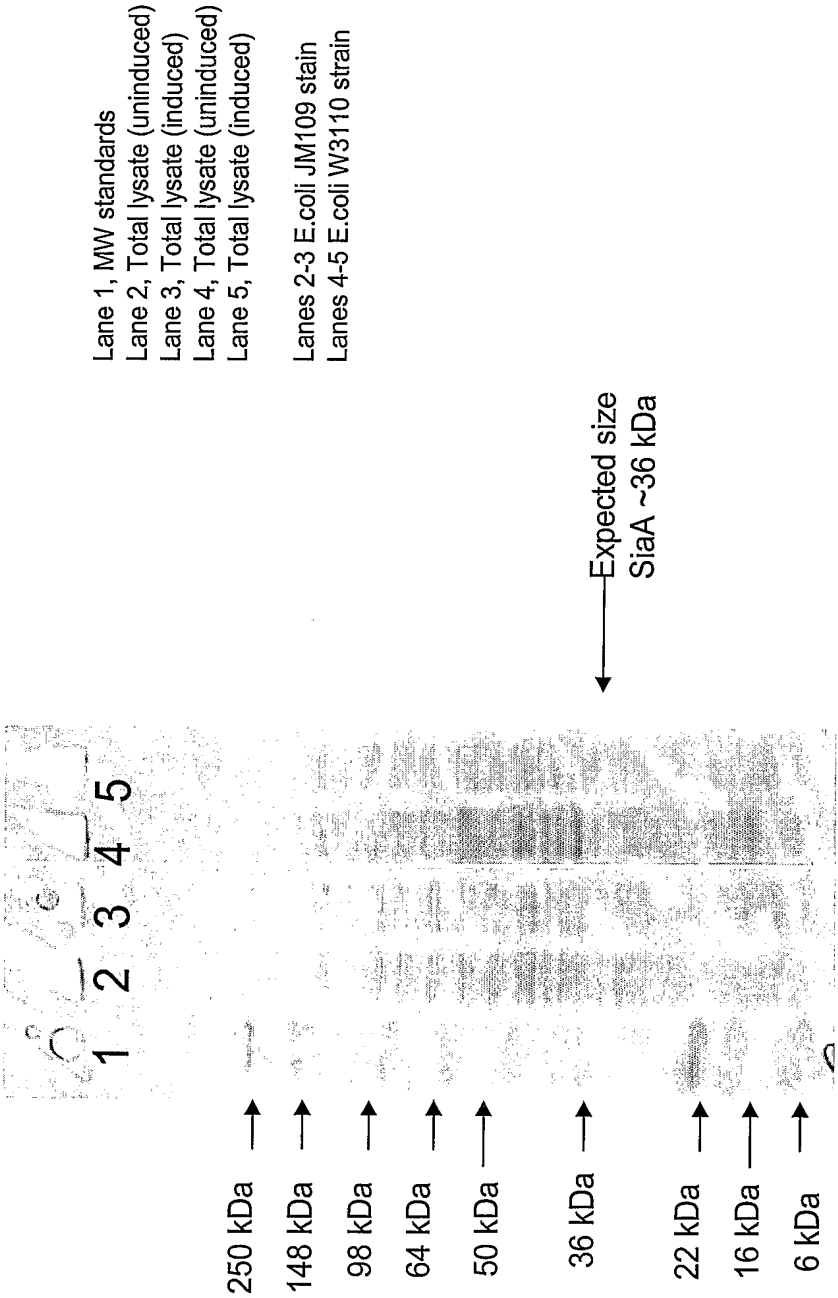
B.



Lanes:  
 1) See Blue Plus 2  
 2) Control enzymes 0hrs  
 3) Control enzymes 16hrs  
 4) Separate refold 0hrs  
 5) Separate refold 16hrs  
 6) Co-refold 0hrs  
 7) Co-refolded 16hrs

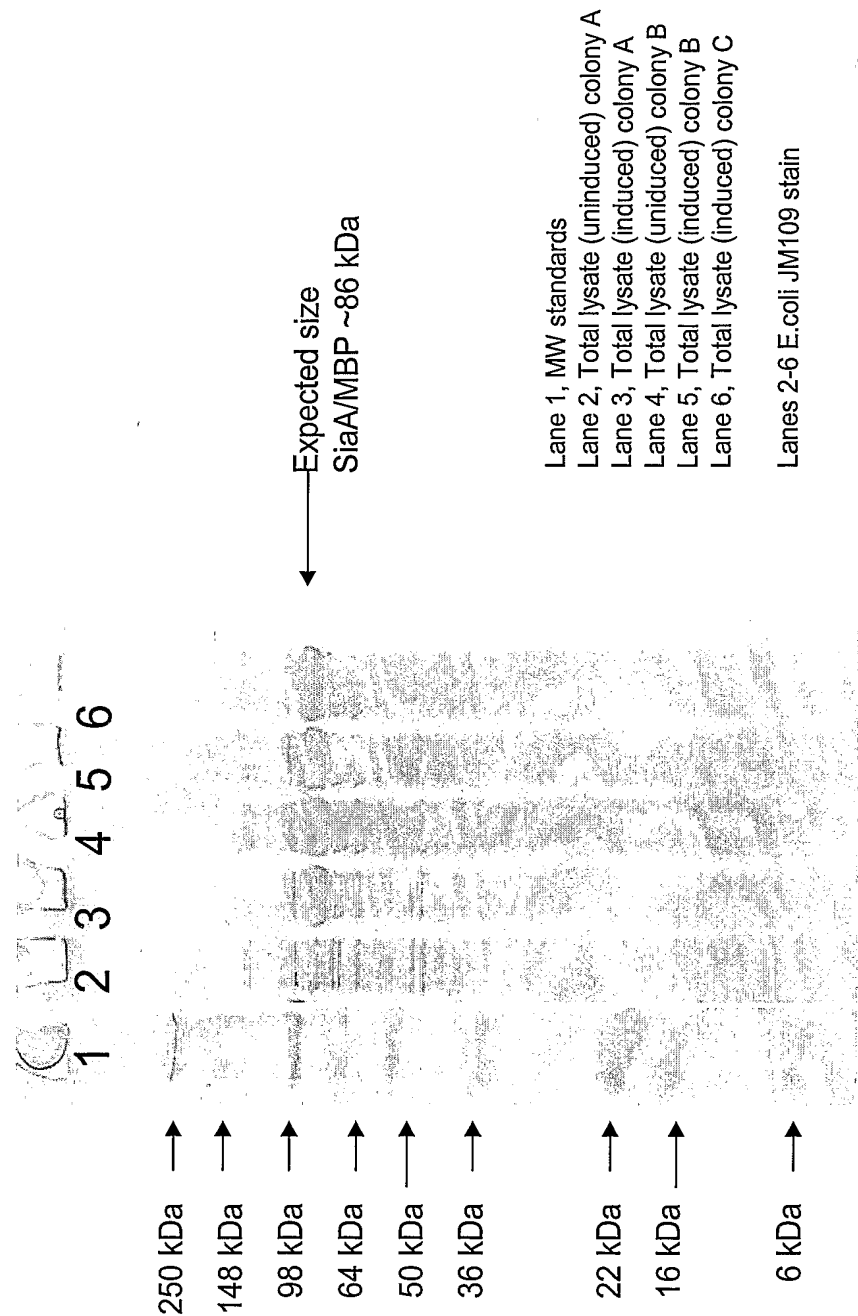


Figure 28: Induced SiaA expression in E.coli



There is no obvious inducible band at the expected  
Mass of 36 kDa for the native SiaA protein.

Figure 29: Induced SiaA/MBP expression in E.coli



High level production of SiaA/MBP even in absence of IPTG induction. Compare to figure X where SiaA production is not obvious. The presence of the fusion partner (MBP) drives high levels of expression.

Figure 30

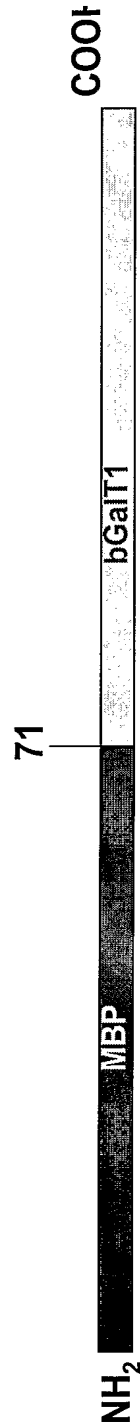
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121 apvpstttrs ltacpeespl lvgpmliefn ipvdlklieq qnpkvklggr ytpmdcisph
181 kvaiiilfrn rgehlkywly ylhpvmvqrqq ldyygiyvinq agesmfmrak llnvvgfkeal
241 kdydyncfvf sdvdlipmnd hntyrdfsqp rhisvamdkf gfsipyvqyf ggvsalskqq
301 flsingfpnn ywgwggeddd iynrlafrgm svsrpnavig kermirhsrd kknepnqgrf
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```

# Bovine (b) GalT1 ( $\beta$ 1,4GalT1) constructs

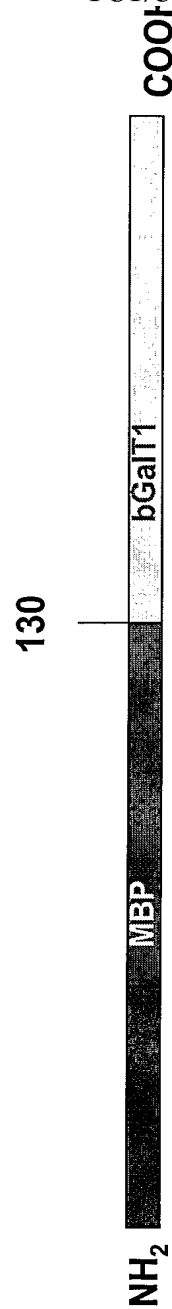
UDP-galactose  $\beta$ -N-acetylglucosaminide  $\beta$ 4-galactosyltransferase (EC 2.4.1.38)



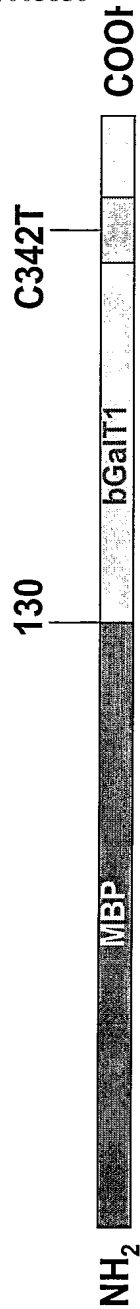
GalT1(40) (S96A+C342T)



MBP-GalT1( $\Delta$ 70)



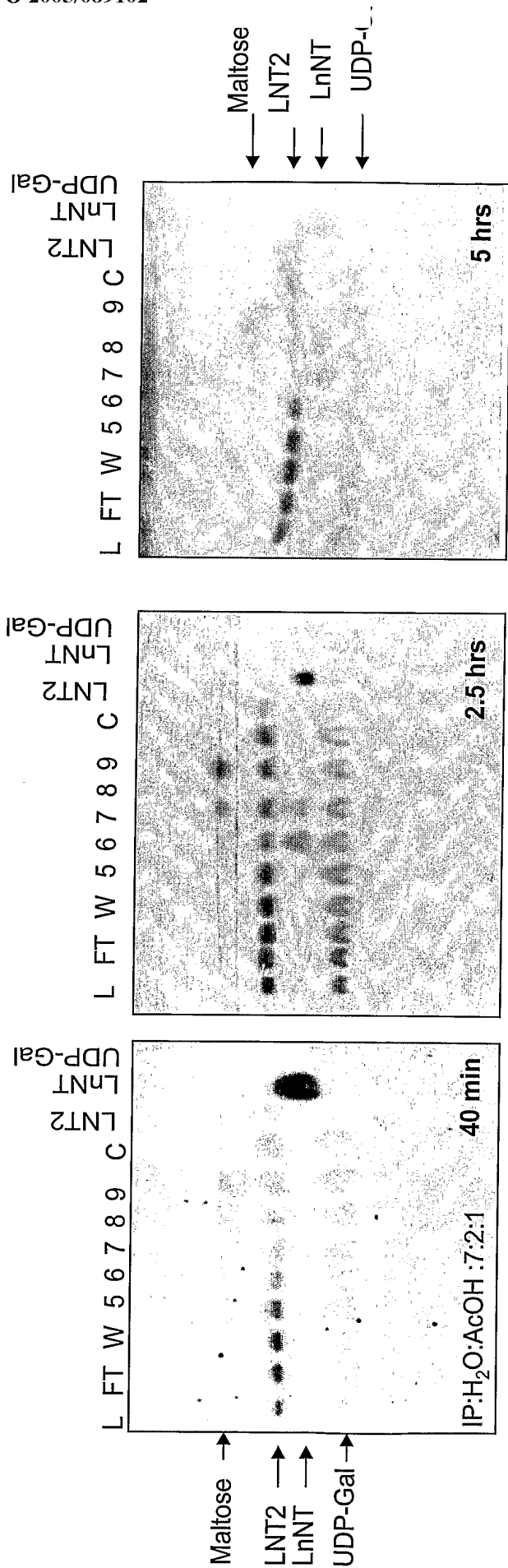
MBP-GalT1( $\Delta$ 129)



MBP-GalT1( $\Delta$ 129) C342T

Figure 31

GaIT1 TLC assay



LnNT reactions with

- L: Loaded sample (refolded, dialyzed MBP-GaIT1(70) onto amylose column)
- FT: Amylose column Flow trough
- W: Amylose column wash
- 5: Maltose Eluted Fr # 5
- 6: Maltose Eluted Fr # 6
- 7: Maltose Eluted Fr # 7
- 8: Maltose Eluted Fr # 8
- 9: Maltose Eluted Fr # 9
- C: Control with water

Figure 32

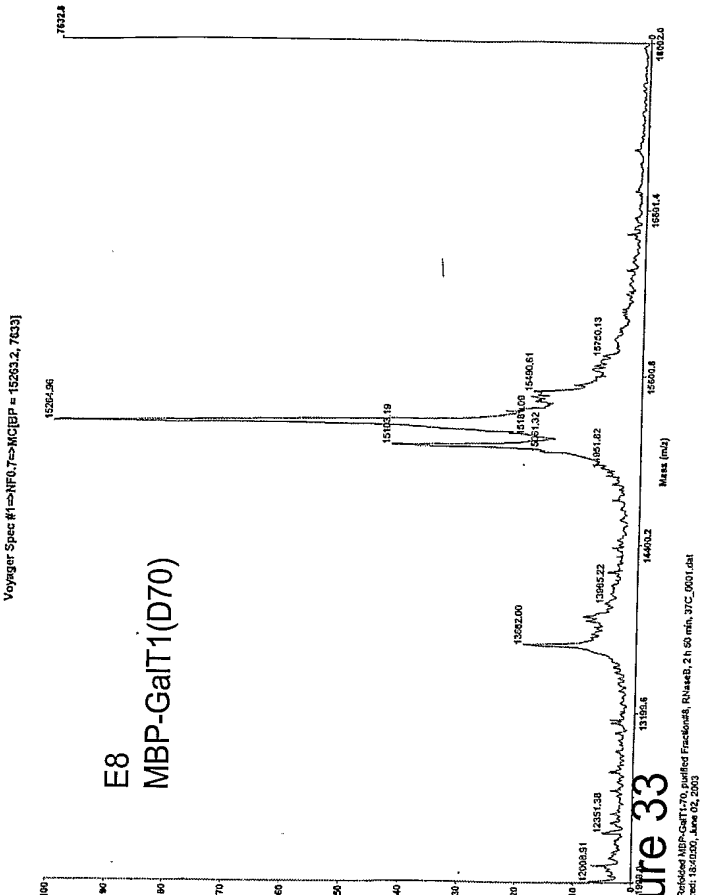
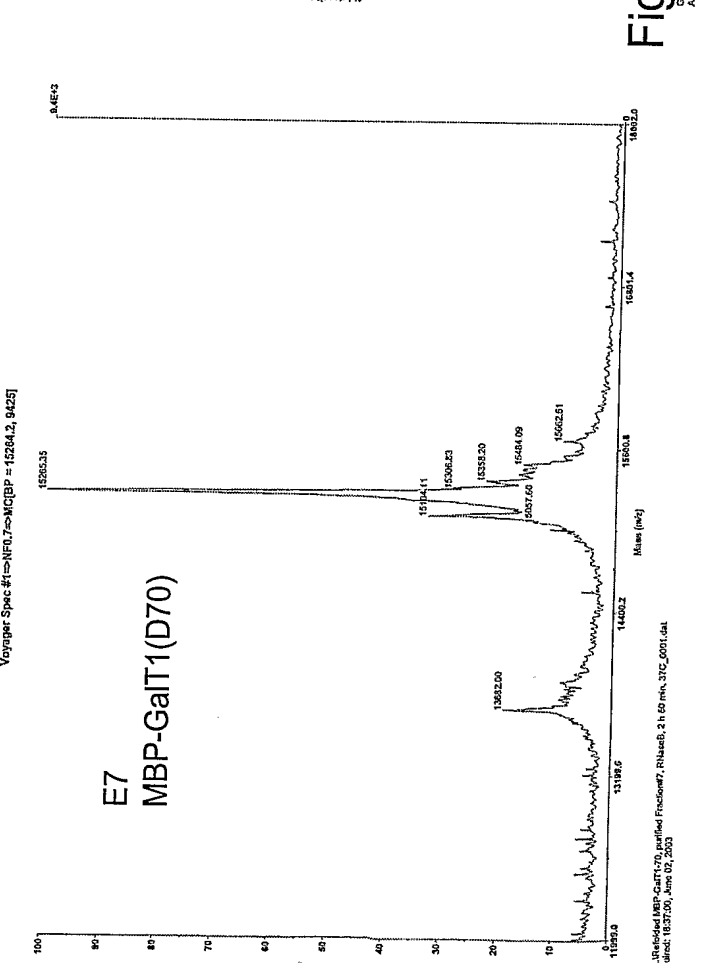
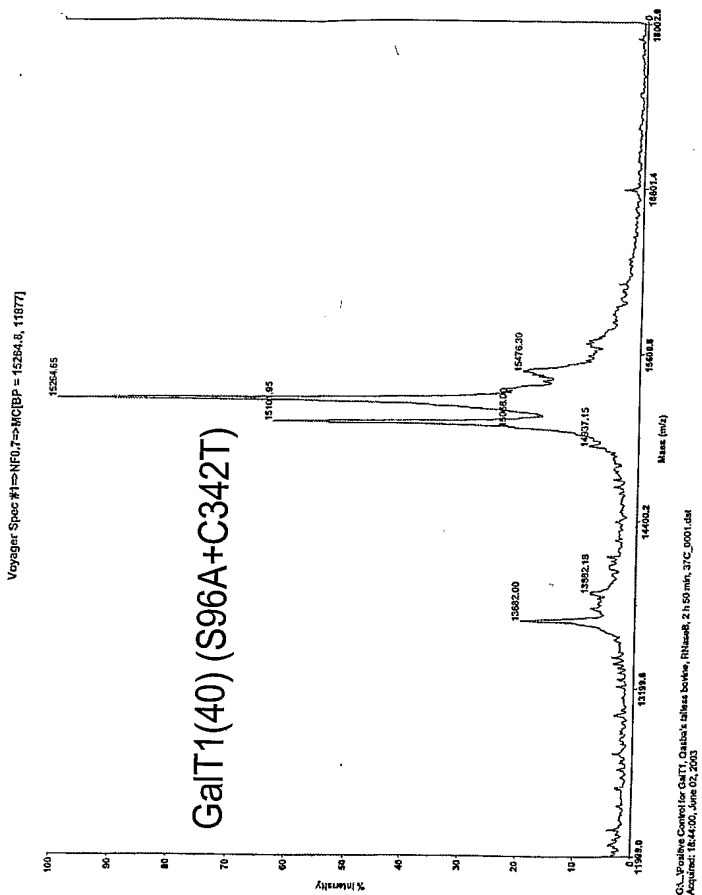
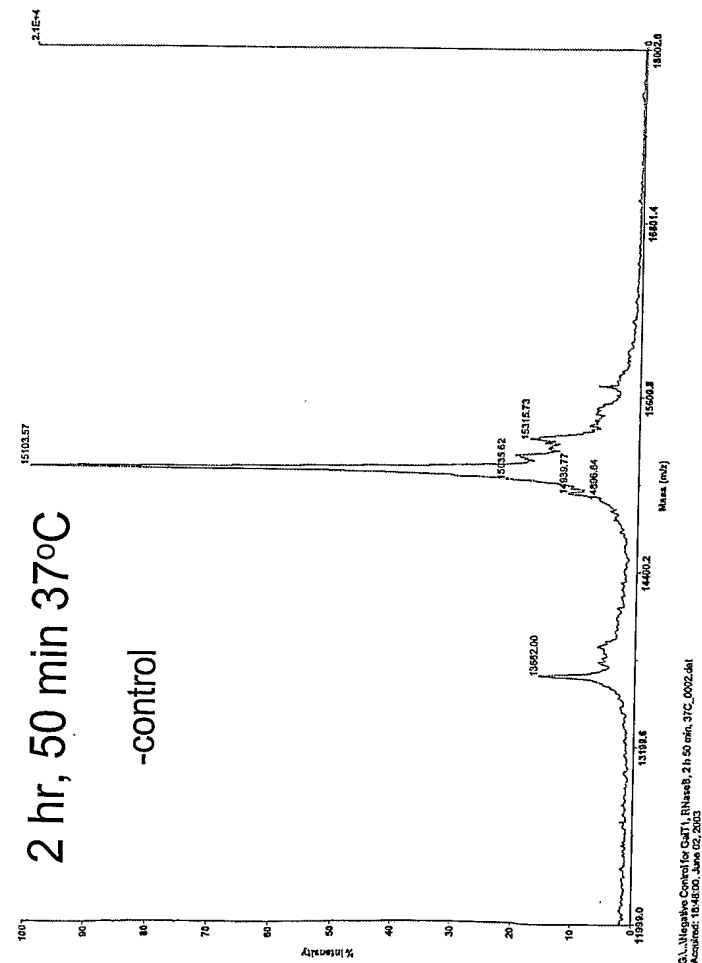


Figure 33

Kinetics of RNase B modelling with GalT1 6.5.03

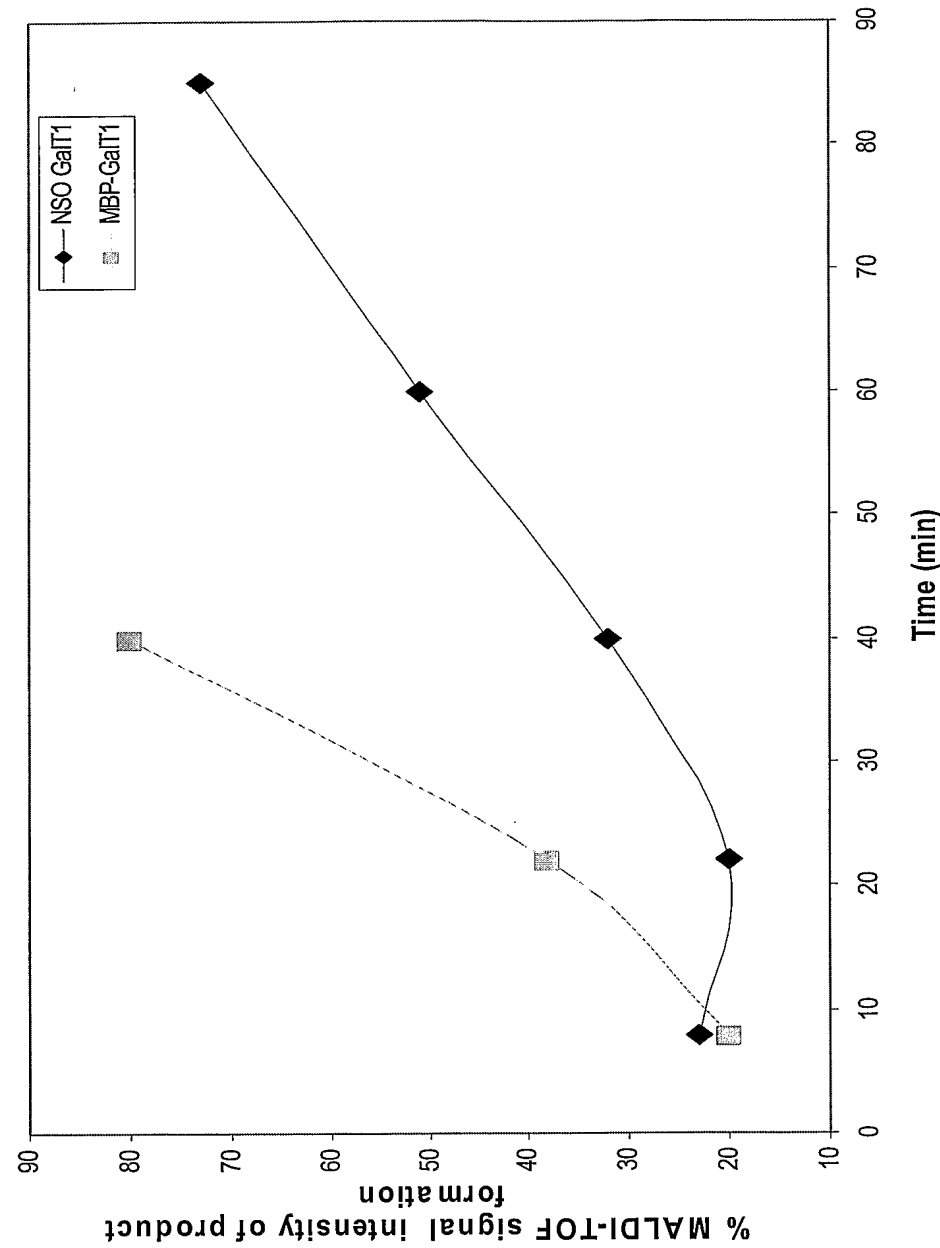
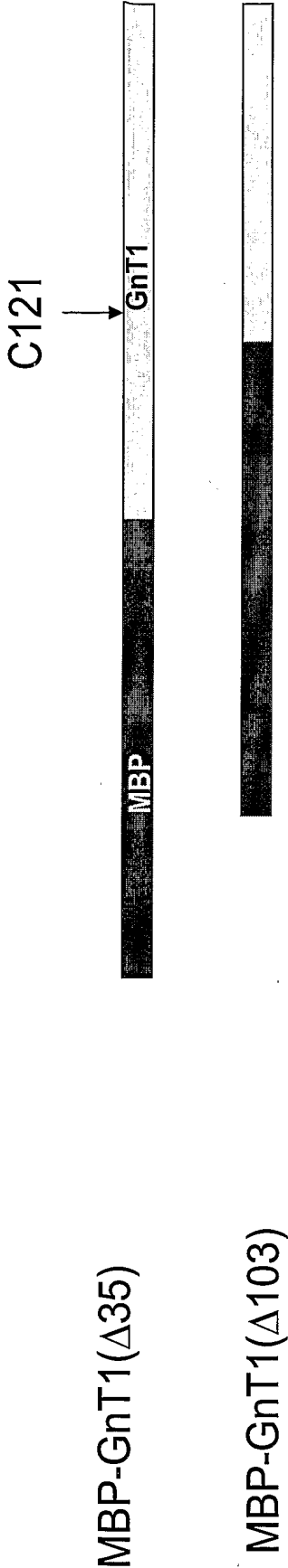


Figure 34

GnT1 constructs

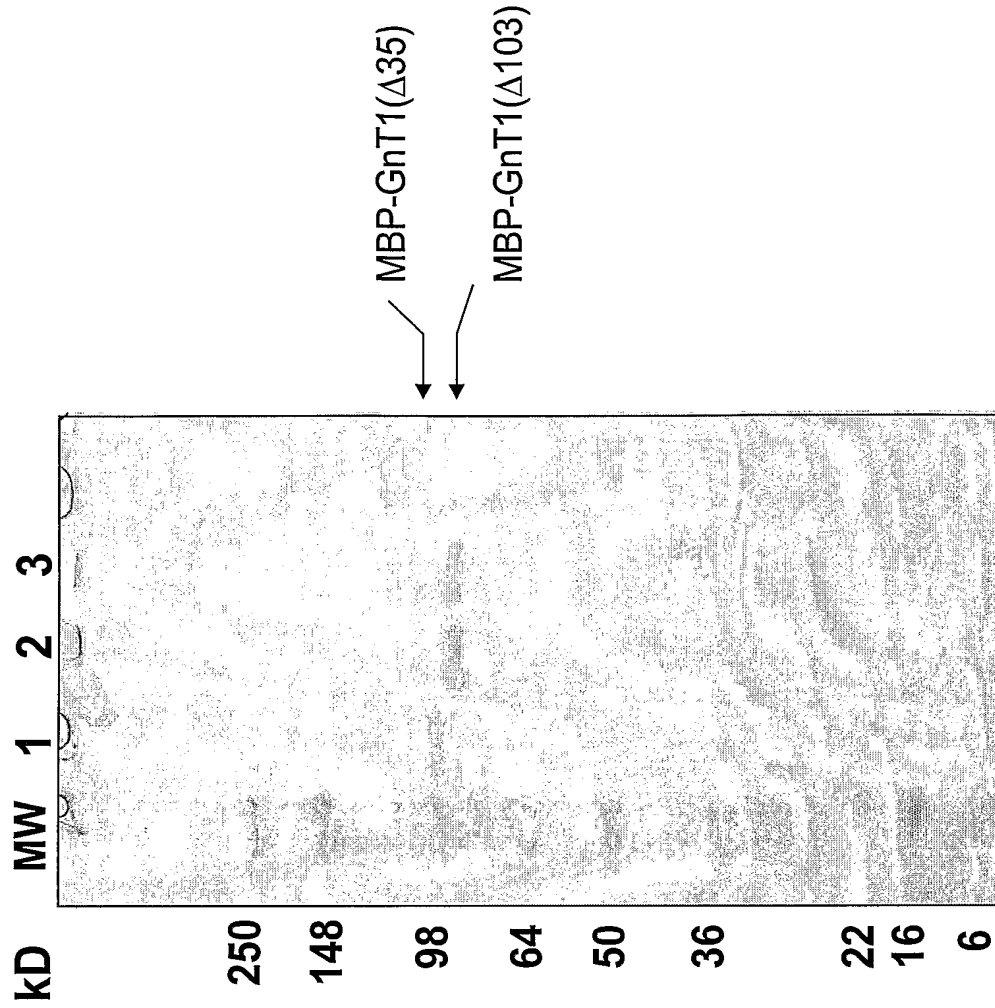


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121 clldkllhyrp saelfpiivs qdcgheetaq aiasygsavt hirqpdlssi avppdhrkfq  
181 gyykiarhyr walgqvfrqf rfpaavvved dlevapdffe yfratypllk adpslwcvs  
241 wndngkeqmv dasrpellyr tdffpoglwl llaelwaele pkwpkafwdd wmrpegrqg  
301 racirpeisr tmtfgrkgvs hgqffdqhlk fiklnqgfvlq ftqldlsylq reaydrdfla  
361 rvygapqlqv ekvrtndrke lgevrvqytg rdsfkafaka lgvmdlksk vpragyrgiv  
421 tfqfrrgrvh lappptwegy dpswn

Figure 35



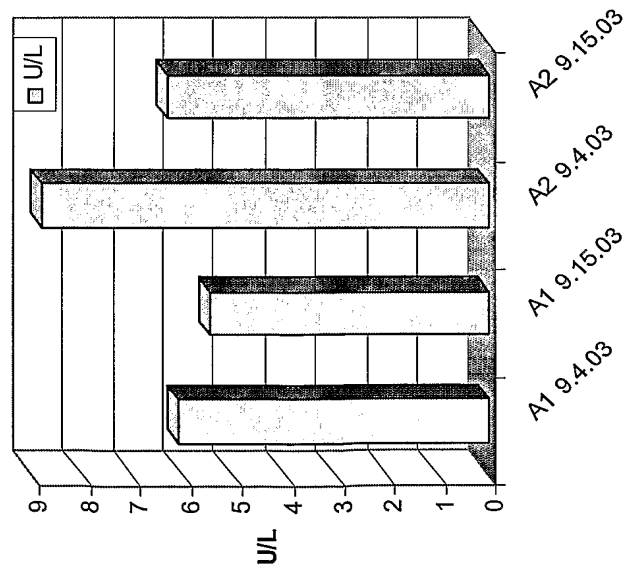
# Comparison of refolded $\Delta 103$ and $\Delta 35$ truncations of MBP-GnT1



- 1- MBP-GnT1( $\Delta 35$ ) C121A
- 2- MBP-GnT1( $\Delta 103$ ) R120A + C121H
- 3- MBP-GnT1( $\Delta 103$ ) C121A

SS 636-49

## Activities of Two different batches (A1 and A2) of Refolded\* MBP-GnT1( $\Delta 35$ ) C121A at different time points



SS 636-127

Figure 36

Figure 37

```
1 mapmrkkstl klltllvlfi fltsfflnys htvvttawfp kqmvielsen fkklmkypyr
61 pctctrciee qrvsawfder fnrsmqpllt aknahleedt ykwwlrlqre kqpnnlndti
121 relfqvvp gn vdp llekrlv scrrcavvgn sgnlkesyyg pqidshdfvl rmnkaptgef
181 eadvgsktth hfvy pesfre laqevsmilv pfktt dleww isattt gtis htyvpvpaki
241 kvkkekil iy hpafiky vfd rwlqghgryp stgilsvifs lhcdevdly gfgadskgnw
301 hhywennpsa gaf rktgvhd gdfesnvtti lasinkirif kgr
```

Figure 38

## A. Human ST6GalNAcI

MRSCWRCRHL SQGVQWSLLAVLVFFLFALPSFIKEPQTKPSRHQRTENIKERSLQS  
 LAKPKSQAPTRARRTTIYAEPVPENNALNTQTQPKAHTTGDRGKEANQAPPEEQDK  
 VPHTAQRAAWKSPEKEKTMVNTLSPRGQDAGMASGRTEAQSWKSQDTKTTQGNQ  
 GQTRKLTASRTVSEKHQGKAATTAKTLIPKSQHRMLAPTGA VSTRTRQKGVTTAVIP  
 PKEKKPQATPPPAPFQSPTTQRNQRLKAANFKSEPRWDFEEKYSFEIGGLQTTCPDSV  
 KIKASKSLWLQKLFLPNLTLFLDSRHFNQSEWDRLEHFAPPFGFMELNYSLVQKVVT  
 RFPPVPQQQLLASLPAGSLRCITCAVVGNGGILNNSHMGQEIDSHDYVFRLSGALIK  
 GYEQDVGTRTSFYGFTAFLSTQSLLILGNRGFKNVPLGKDVRYLHFLEGTRDYEWE  
 ALLMNQTVMSKNLFWFRHRPQEAFFREALHMDRYLLHPDFLR YMKNRFLRSKTL D  
 GAHWRIYRPTTGALLLLTALQLCDQVSAYGFITEGHERFSDHYDTSWKRLIFYINH  
 DFKLEREVWKRLHDEGIIRLYQRPGPGTAKAKN

## B. Chicken ST6GalNAcI

MGFLIRRLPKDSRIFRWLLILTVFSFITSFSALFGMEKSIFRQLKIYQSI AHMLQVDTQ  
 DQQGSNYSANGRISKVGLERDIAWLELNTAVSTPSGEGKEEQKKTVPVAKVEEAK  
 EKVTVPKPFPEVMGITNTTASTASVVERTKEKTTARPVPGVGEADGKRTTIALPSMKE  
 DKEKATVKPSFGMKVAHANSTSKDKPKAEPPASVKAIRPVTQAATVTEKKKLRAA  
 DFKTEPQWDFDDEYILDSSSPVSTCSESVRAKAAKSDWLRDLFLPNITL FIDKSYFNV  
 SEWDRLEHFAPPYGFMEELNYSLVEEVMSRLPPNPHQQQLLLANSSSNVSTCISCAVVG  
 NGGILNNSGMGQEIDSHDYVFRVSGAVIKGYEKDVGT KTSFYGFTA YSLVSSLQNLG  
 HKGFKKIPQGKHRYIHFLEAVRDYEWL KALLLDKDIRKGFLNYYGRRPRERFDEDF  
 TMNKYLVAHPDFLR YLKNRFLKSKNLQKPYWRLYRPTTGALLLLTALHLCDRVSAY  
 GYITEGHQKYS DHYDKEWKRLVFYVNHD FNLEKQVWKRLHDENIMKLYQRS

## C. Mouse ST6GalNAcI protein beginning at residue 32 of the native mouse protein

DPRAKDSRCQFIWKNDASAQENQQAEPQVPIMTLSPRVHNKESTSVSSKDLKKQER  
 EAVQGEQAEGKEKRKLETIRPAPENPQSKAEPAAKTPVSEHLDKLPRTPGALSTRKTP  
 MATGAVPAKKKVQATKSPASSPHPTTRRRQRLKASEFKSEPRWDFEEYSLDMSSL  
 QTNCASV KIKASKSPWLQNIFLPNITLFLDSGRFTQSEWNRLEHFAPPFGFMELNQSL  
 VQKVVTFRPPVRQQQLLASLP TGYSKCITCAVVGNGGILNDSRVGREIDSHDYVFR  
 LSGAVIKGYEQDVGTRTSFYGFTAFLSTQSILILGRRGFQHVPLGKDVRYLHFLEGTR  
 NYEWLEAMFLNQTLAKTHLSWFRHRPQEAFFRNALDLDRYLLHPDFLR YMKNRFL  
 RSKTLDTAHWRIYRPTTGALLLLTALHLCDKVSAYGFITEGHQRFS DHYDTSWKRL  
 IFYINHDFRLERMVWKRLHDEGIWLYQR PQSDKAKN

Hum (h)ST6GalNAcI  
truncations

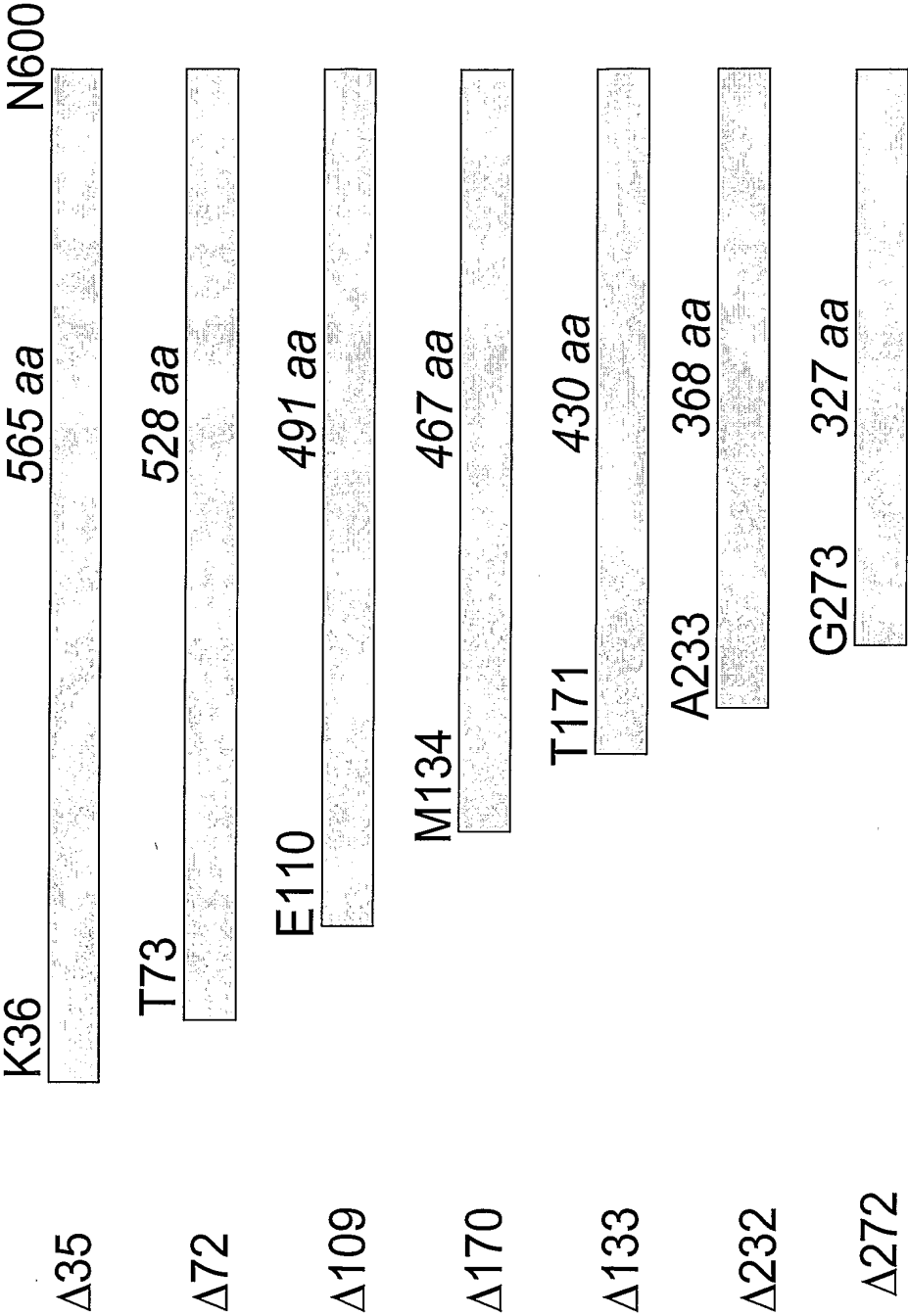


Figure 39

# MBP-hST6GalNAc constructs

WO 2005/089102

PCT/US2005/003856

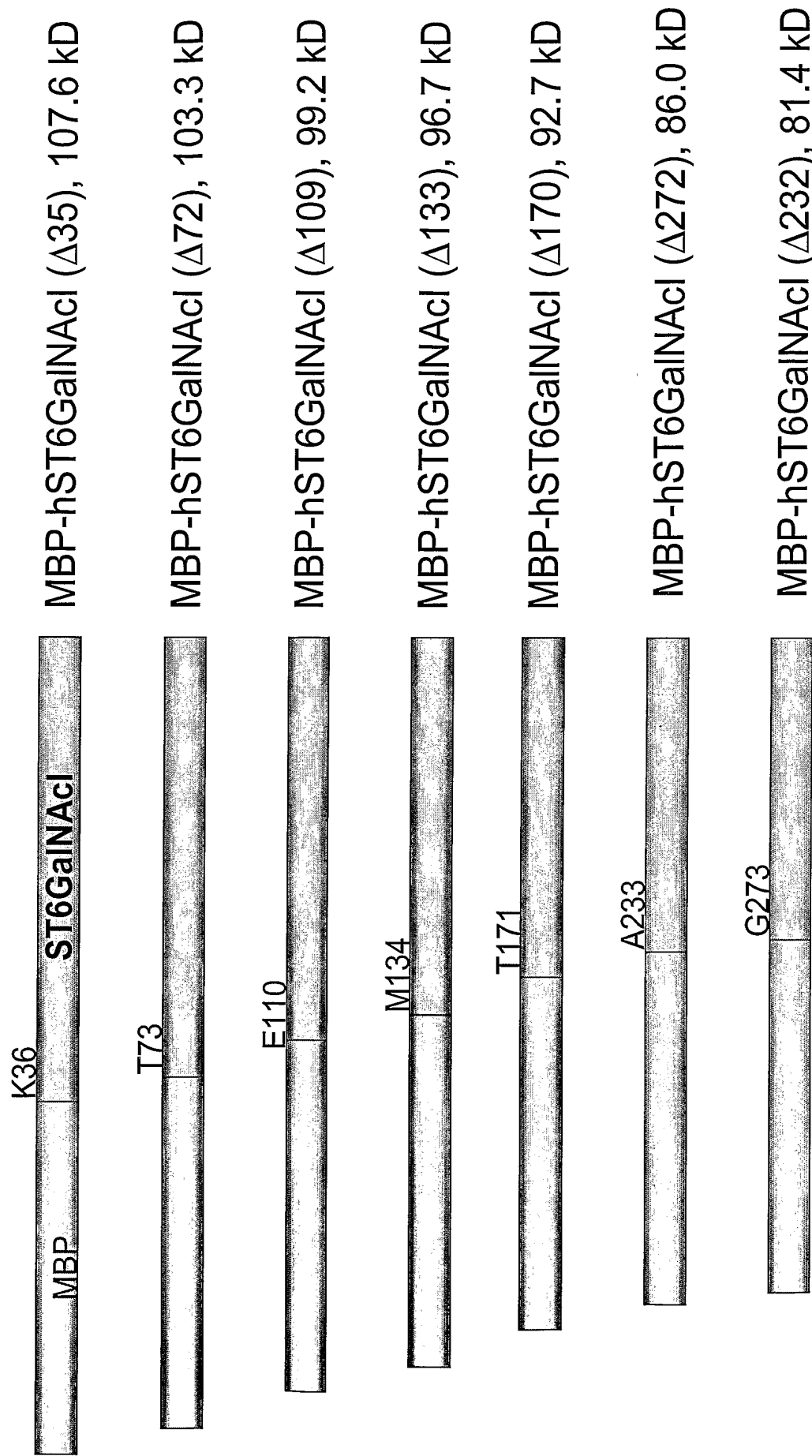


Figure 40

Figure 41

MASKSWLNFLTFLCGSAIGFLLCSQLFSILLGEKVDTQPNVLHNDPHARHSDDNGQN  
HLEGQMNFNADSSQHKDENTDIAENLYQKVRILCWVMTGPQNLEKKAKHV KATW  
AQRCNKVLFMSSEENKDFPAVGLKTKEGRDQLYWKTIKAFQYVHEHYLEDADWFL  
KADDDTYVILDNLRWLLSKYDPEEPIYFGRRFKPYVKQGYMSGGAGYVLSKEALKR  
FVDAFKTDKCTHSSSIEDLALGRCMEIMNVEAGDSRDTIGKETFHFPVPEHHLIKGYL  
PRTFWYWNYNYYPPVEGPGCCSDLAVSFHYVDSTTMYELEYLVYHLRPYGYLYRY  
QPTLPERILKEISQANKNEDTKVKLGNP

Sequence	Size	Range		Mode						
SC1GALT1.AMI	342	1-		342 Normal						
SPTS122.AMI	342	1-		342 Normal						
		10	20	30	40	50				
SC1GALT1.AMI	1	EFMPYDGH RH	GDVNDAHSH	DMMEMSGPEQ	DVGGHEHVHE	NSTIAERLYS				
SPTS122.AMI	1	EFMPYDGH RH	GDVNDAHSH	DMMEMSGPEQ	DVGGHEHVHE	NSTIAERLYS				
		60	70	80	90	100				
SC1GALT1.AMI	51	EVRLVCWIMT	NPSNHQKKAR	HVKRTWGKRC	NKLIFMSSAK	DDELDAVALP				
SPTS122.AMI	51	EVRLVCWIMT	NPSNHQKKAR	HVKRTWGKRC	NKLIFMSSAK	DDELDAVALP				
		110	120	130	140	150				
SC1GALT1.AMI	101	VGEGRNNLWG	KTKEAYKYIY	EHINDADWF	LKADDDTYTI	VENMRYMLYP				
SPTS122.AMI	101	VGEGRNNLWG	KTKEAYKYIY	EHINDADWF	LKADDDTYTI	VENMRYMLYP				
		160	170	180	190	200				
SC1GALT1.AMI	151	YSPETPVYFG	CKFKPYVKQG	YMSGGAGYVL	SREAVRRFV	EALPNPKLCK				
SPTS122.AMI	151	YSPETPVYFG	CKFKPYVKQG	YMSGGAGYVL	SREAVRRFV	EALPNPKLCK				
		210	220	230	240	250				
SC1GALT1.AMI	201	SDNSGAEDVE	IGKCLQNVNV	LAGDSRDSNG	RGRFFPFVPE	HHLIPSHTDK				
SPTS122.AMI	201	SDNSGAEDVE	IGKCLQNVNV	LAGDSRDSNG	RGRFFPFVPE	HHLIPSHTDK				
		260	270	280	290	300				
SC1GALT1.AMI	251	KFWYWQYIFY	KTDEGLDCCS	DNAISFHYVS	PNQMYVLDYL	TYHLRPYGII				
SPTS122.AMI	251	KFWYWQYIFY	KTDEGLDCCS	DNAISFHYVS	PNQMYVLDYL	TYHLRPYGII				
		310	320	330	340	350				
SC1GALT1.AMI	301	NTPDALPNKL	AVGELMPEIK	EQATESTSDG	VSKRSAETKT	Q*.....				
SPTS122.AMI	301	NTPDALPNKL	AVGELMPEIK	EQATESTSDG	VSKRSAETKT	Q*.....				

Figure 43

A. *Yersinia* MBP

MKIEEGKLVI	WINGDKGYNG	LAEVGKKFEK	DTGIKVTIEH	PDKLEEKFPQ
VAATGDGPDI	IFWAHDRFGG	YAQSGLLAEL	TPSKAFQEK	FPFTWDVAVRF
NGKLIGYPVA	VEALSLIYNK	DLVKEAPKTW	EEIPALDKTL	RANGKSAIMW
NLQEPYFTWP	VIAADGGYAF	KFENGVIDAK	NVGVNNAGAQ	AGLQFIVDLV
KNKHINADTD	YSIAEAAFNK	GETAMTINGP	WAWSNIDKSK	INYGVTLLPT
FHGQPSKPFV	GVLTAGINAA	SPNKELATEF	LENYLITDQG	LAEVNKKDKPL
GAVALKSFEQ	QLAKDPRIAA	TMDNATNGEI	MPNIPQMAAF	WYATRSVAVLN
AITGRQTVEA	ALNDAATRIT	K		

B. *E. coli* MBP

MKIEEGKLVI	WINGDKGYNG	LAEVGKKFEK	DTGIKVTVEH	PDKLEEKFPQ
VAATGDGPDI	IFWAHDRFGG	YAQSGLLAEL	TPDKAFQDKL	YPFTWDVAVRY
NGKLIAYPIA	VEALSLIYNK	DLLPNPPKTW	EEIPALDKEL	KAKGKSALMF
NLQEPYFTWP	LIAADGGYAF	KYENGKYDIK	DVGVDNAGAK	AGLTFLVDLI
KNKHMNADTD	YSIAEAAFNK	GETAMTINGP	WAWSNIDTSK	VNYGVTVLPT
FKGQPSKPFV	GVLSAGINAA	SPNKELAKEF	LENYLLTDEG	LEAVNKKDKPL
GAVALKSYEE	ELAKDPRIAA	TMENAQKGEI	MPNIPQMSAF	WYAVRTAVIN
AASGRQTVDE	ALKDAQTNS			

C. *Pyrococcus furiosus* MBP

MKIEEGKVVI	WHAMQPNELE	VFQSLAE EYM	ALSPEVEIVF	EQKPNLEDAL
KAAIPTGQGP	DLFIWAHDWI	GKFAEAGLLE	PIDEYVTEDL	LNEFAPMAQD
AMQYKGHYIA	LPFAAETVAI	IYNKEMVSEP	PKTFDEMKA	MEKYYPDANE
KYGIAPWPIA	YFISAIQAFA	GGYYFDDKTE	QPGLDKPETI	EGFKFFFTETI
WPYMAPTGDY	NTQQSIFLEG	RAPMMVNGPW	SINDVKKAGI	NFGVVPLPPI
IKDGKEYWPR	PYGGVKLIYF	AAGIKNKDAA	WKFAKWLTT	EESIKTALAE
LGYIPVLTKV	LDDPEIKNDP	VIYGFQAVQ	HAYLMPKSPK	MSAVWGGVDG
AINELQDPQ	NADIEGILKK	YQQEILNNMQ	G	

D. *Thermococcus litoralis* MBP

MKIEEGKIVF	AVGGAPNEIE	YWKGVIAEFE	KKYPGVTVEL	KRQATDTEQR
RLDLVNALRG	KSSDPDVFLM	DVAWLQGFIA	SGWLEPLDDY	VQKDNLDLSV
FFQSVINLAD	KQGGKLYALP	VYIDAGLLYY	RKDLLEKYGY	SKPPETWQEL
HEMAQKIQSG	ERETNPFWG	FVWQKGQYEG	LVCDFVEYVY	SNGGSLGEFK
DGKWVPTLNK	PENVEALQFM	VDLIHKYKIS	PPNTYTEMTE	EPVRLMFQQG
NAAFERNWPY	AWGLHNADDS	PVKGKGVGAP	LPHFPGHKS	ATLGGWHIGI
SKYSDNKALA	WEFVKFVESY	SVQKGFAMNL	GWNPGRVDVY	DDPAVVS KSP
HLKELRAVFE	NAVPRPIVPY	YPQLSEIIQK	YVNSALAGKI	SPQEALDKAQ
KEAEELVKQY	SK			

E. *Thermatoga maritima* MBP

MKIEQTKLTI	WSSEKQVDIL	QKLGEFEKAK	YGIPVEVQYV	DFGSIKSKFL
TAAPQGQGAD	IIVGAHDWVG	ELAVNGLIEP	IPNFSDLKNF	YDTALKAFSY
GGKLYGVPIA	MEAVALIYNK	DYVDSVPKTM	DELIEKAKQI	DEEYGGVEVRG
FIYDVANFYF	SAPFILGYGG	YVFKETPQGL	DVTDIGLANE	GAVKGAKLIK
RMIDEGVLTP	GDNYGTMDSM	FKEGLAAMII	NGLWAIKSYK	DAGINYGVAP
IPELEPGVPA	KPFVGVQGM	INAKSPNKVI	AMEFLT NFIA	RKETMYKIYL



ADPRLPARKD VLELVKDNDP VVAFTQSASM GTPMPNVPEM APVWSAMGDA  
LSIIINGQAS VEDALKEAVD KIKAQIEK

*F. Vibrio cholerae* MBP

MKIEEGQLTI WINGDKGYNG LAEVGKKFEA DTGIKVTVAH PDALQDKFPQ  
TAATGDGPDI VFWAHDRFGG YAEAGLLVEI KPSAKIQEGI VDFAWDAVKY  
NGKIIGYPPIA VESLSLIYNK DLVPNPPKSW EEVAELDAKL KKEGKSAIMW  
NLKEPYFTWP LMAADGGYAF KYGVDGYDVK DAGINNKGVK DAMNFVKGLV  
DKGVISPDMD YSVSESAFNQ GNTAMTINGP WSWGNIKESG INYGVTTLPK  
FNGQASKPFV GVLTAGISTA SPNKDLAVEF IENYLLTNDG LRMVNNDKPL  
GAVALNSFQR ELDADARIAA TMDNAMNGEI MPNIPQMNAF WSSAKNAIIN  
IVDGRQTVDA ALADAEKQMT KP

Fig 43 2/2

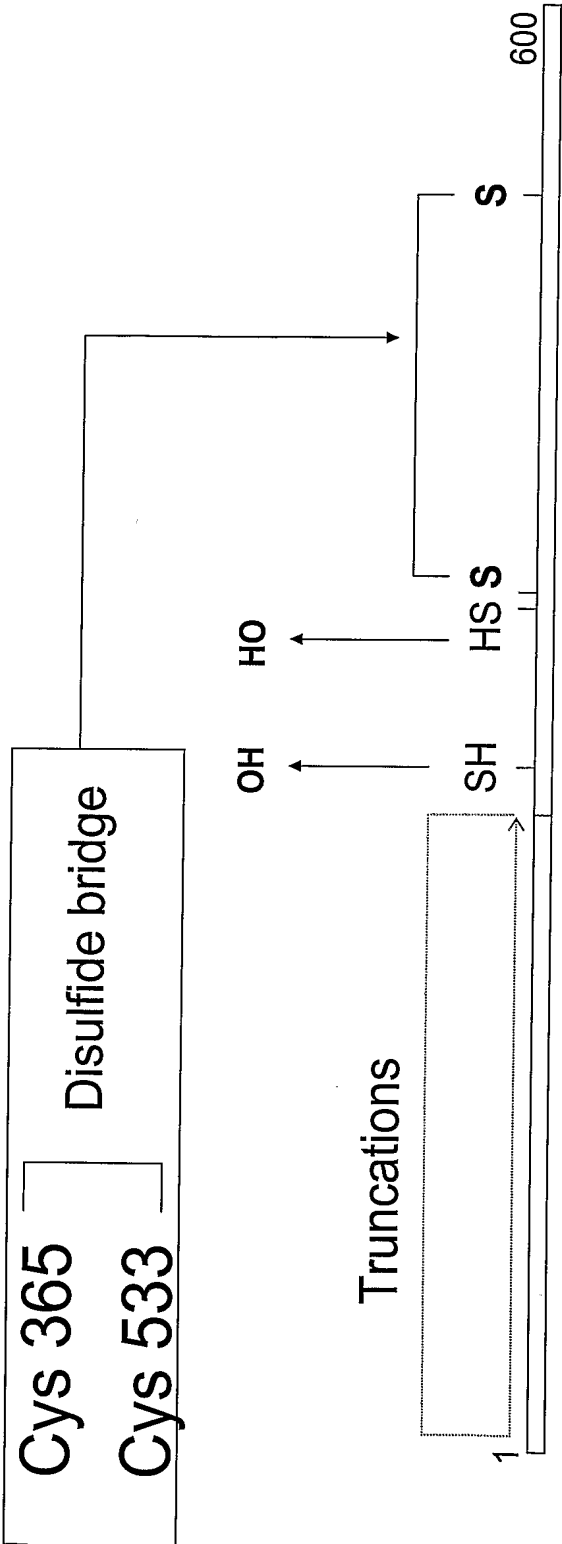
Figure 44

		1		50
HSGALNAT1.pep	(1)	MRKFAYCKVVLATSLTWVLLDMELLLYFS-----	ECNKC	
HSGALNAT2.pep	(1)	MRRRS--RMLLCFAFLWVLGIAYMYSGGGSALAGGAGGGAGRKEDWNEI		
Consensus	(1)	MRK A KMLL A IWL F L	D N	
		51		100
HSGALNAT1.pep	(35)	DEKKERGLPAGDVLEPVQKPHEGP-G-----EMGKPVVTPKEDQEKMKEM		
HSGALNAT2.pep	(49)	DPIKKKDLHHSNGEEKAQSMETLPPGKVRWPDFNQEAYVGGTMVRSGODP		
Consensus	(51)	D K K L E Q P G D I D		
		101		150
HSGALNAT1.pep	(79)	FKINQFNLMASEMIALNRSLPDVRLGCKTKVMPDNLPTTSVVIVFHNEA		
HSGALNAT2.pep	(99)	YARNKFNOVESDKLRMDRAIPDTRHDQCQRKQWRVDLPATSVVITFHNEA		
Consensus	(101)	F N FN M SD I L RAIPD R D C K W LP TSVVI FHNEA		
		151		200
HSGALNAT1.pep	(129)	WSTLLRTVHSVLRSPRHMEETLVDDASERDFLKRPLESYVKKLKVPV		
HSGALNAT2.pep	(149)	RSALLRTVSVSVLKSPPHLTKETLVDDYSN-----DPEDGALLGKIEKV		
Consensus	(151)	S LLRTV SVI KSP HLI EILVDD S P D L V		
		201		250
HSGALNAT1.pep	(179)	HVIRMEQRSGLIRARLKGAAVSKGQVITFLDAHCECTVGWLEPLLARIKH		
HSGALNAT2.pep	(194)	RVLNRDRREGLMRSRVGADAAQAKVITFLDSHCENEHWLEPLLERRVAE		
Consensus	(201)	VIR D R GLIRARLKGA A A VITFLDAHCEC WLEPLL RI		
		251		300
HSGALNAT1.pep	(229)	DRRTVVCPIIDVISDDTFEYMAGSDMTYGGFNWKLNRWYPVPQREMDRR		
HSGALNAT2.pep	(244)	DRTRVVSPIIDVINMDNFQYVGASADLKGGFDWNLVFKWDYMTPEQRRSR		
Consensus	(251)	DR VV PIIDVI D F YMAAS GGF W L FKW M R		
		301		350
HSGALNAT1.pep	(279)	KGDRTLPVRTPTMAGGLFSIDRDYFQETGYDAGMDTWGGENLEISFRVW		
HSGALNAT2.pep	(294)	QGNPVAPLKTPTMAGGLFVMDKIFYFEELGKYDMMMDVWGGENLEISFRVW		
Consensus	(301)	G PIKTP IAGGLF IDK YF EIG YD MDIWGGENLEISFRIV		
		351		400
HSGALNAT1.pep	(329)	QCGGTLEIVTCSHVGHVFRKATPYTFPGGTGQTINKNNRRRLAEVWMDEFK		
HSGALNAT2.pep	(344)	QCGGSLEIIPC SRVGHVFRKQHPYTFPGGSGTVFARNTRRAAEVWMDEYK		
Consensus	(351)	QCGGSLEII CS VGHVFRK PYTFPGGSG I KN RR AEVWMDEFK		
		401		450
HSGALNAT1.pep	(379)	NFFYIISPGVTKVDYGDISSRVGLRHKLQCKPFSWYLENIYPDSQIPRHY		
HSGALNAT2.pep	(394)	NFFYYAAVPSARNVPYGNIQSRLELRKKLSCKPFWYLENVYPRLRVPDHQ		
Consensus	(401)	NFFY P V YG I SRL LR KL CKPF WYLENIYPD IP H		
		451		500
HSGALNAT1.pep	(429)	FSLGEIRNVETNQCLDNMARKENKVGLENCHGMGGNQVESYTANKEIRT		
HSGALNAT2.pep	(444)	DIAFGALQQGTN-CLDTLGHFADGVVGVYECHNAGGNQEWALTKEKSVKH		
Consensus	(451)	N TN CLD LA VGIF CH GGNQ FA T K IK		
		501		550
HSGALNAT1.pep	(479)	DDLCLDVSKLN--GPVTMLKCHHLKGNQLWEYDPVKLTQLQHVNSNQCLDK		
HSGALNAT2.pep	(493)	MDLCLTVVDRAPGSLTKLQGCRENDSROKWEQIEGNSKL RHVGSNLC LDS		
Consensus	(501)	DLCL V I L C Q WE L HV SN CLD		
		551		584
HSGALNAT1.pep	(527)	ATEEDSQVPSIRDCNGSRSQQWLLRNVTLP EIF-		
HSGALNAT2.pep	(543)	RTAK-SGGLSV EVC GPALSQQWKFTLN LQQ----		
Consensus	(551)	T S SI C A SQW		

**CYSTEINE ENGINEERING IN hum ST6GalNAcI**

Cys 280	→ Ser 280 ( <i>Aat</i> II)
Cys 362	→ Ser 362 ( <i>Bgl</i> II)
Cys 362	→ Thr 362 ( <i>Acc</i> III)

Restriction sites created



<u>Single mutants:</u>	C280S, C362S, C362T
<u>Double mutants:</u>	[C280S + C362S], [C280S + C362T]

Figure 45